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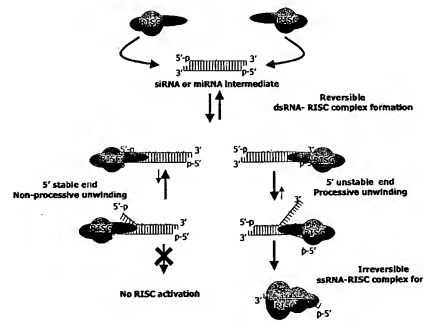
(54) Title: FUNCTIONAL AND HYPERFUNCTIONAL SIRNA



(57) Abstract: Efficient sequence specific gene silencing is possible through the use of siRNA technology. By selecting particular siRNAs by rationale design, one can maximize the generation of an effective gene silencing reagent, as well as methods for silencing genes.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Functional and Hyperfunctional siRNA

Cross Reference to Related Applications

This application claims the benefit of the filing date of U.S. Provisional Application Serial No. 60/426,137, filed November 14, 2002, entitled "Combinatorial Pooling Approach for siRNA Induced Gene Silencing and Methods for Selecting siRNA," and U.S. Provisional Application Serial No. 60/502,050, filed September 10, 2003, entitled "Methods for Selecting siRNA," the entire disclosures of which are hereby incorporated by reference into the present disclosure.

Field of Invention

The present invention relates to RNA interference ("RNAi").

Background of the Invention

Relatively recently, researchers observed that double stranded RNA ("dsRNA") could be used to inhibit protein expression. This ability to silence a gene has broad potential for treating human diseases, and many researchers and commercial entities are currently investing considerable resources in developing therapies based on this technology.

Double stranded RNA induced gene silencing can occur on at least three different levels: (i) transcription inactivation, which refers to RNA guided DNA or histone methylation; (ii) siRNA induced mRNA degradation; and (iii) mRNA induced transcriptional attenuation.

It is generally considered that the major mechanism of RNA induced silencing (RNA interference, or RNAi) in mammalian cells is mRNA degradation. Initial attempts to use RNAi in mammalian cells focused on the use of long strands of dsRNA. However, these attempts to induce RNAi met with limited success, due in part to the induction of the interferon response, which results in a general, as opposed to a target-specific, inhibition of protein synthesis. Thus, long dsRNA is not a viable option for RNAi in mammalian systems.

More recently it has been shown that when short (18-30 bp) RNA duplexes are introduced into mammalian cells in culture, sequence-specific inhibition of target mRNA can be realized without inducing an interferon response. Certain of these short dsRNAs, referred to as small inhibitory RNAs ("siRNAs"), can act catalytically at sub-molar concentrations to cleave greater than 95% of the target mRNA in the cell. A description of the mechanisms for siRNA activity, as well as some of its applications are described in Provost *et al., Ribonuclease Activity and RNA Binding of Recombinant Human Dicer,* E.M.B.O. J., 2002 Nov. 1; 21(21): 5864–5874; Tabara *et al., The dsRNA Binding Protein RDE-4 Interacts with RDE-1, DCR-1 and a DexH-box Helicase to Direct RNAi in C. elegans,* Cell 2002, June 28;109(7):861-71; Ketting *et al., Dicer Functions in RNA Interference and in Synthesis of Small RNA Involved in Developmental Timing in C. elegans;* Martinez *et al., Single-Stranded Antisense siRNAs Guide Target RNA Cleavage in RNAi,* Cell 2002, Sept. 6; 110(5):563; Hutvagner & Zamore, *A microRNA in a multiple-turnover RNAi enzyme complex,* Science 2002, 297:2056.

From a mechanistic perspective, introduction of long double stranded RNA into plants and invertebrate cells is broken down into siRNA by a Type III endonuclease known as Dicer. Sharp, *RNA interference—2001,* Genes Dev. 2001, 15:485. Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs. Bernstein, Caudy, Hammond, & Hannon, *Role for a bidentate ribonuclease in the initiation step of RNA interference,* Nature 2001, 409:363. The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition. Nykanen, Haley, & Zamore, *ATP requirements and small interfering RNA structure in the RNA interference pathway,* Cell 2001, 107:309. Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleaves the target to induce silencing. Elbashir, Lendeckel, & Tuschl, *RNA interference is mediated by 21- and 22-nucleotide RNAs,* Genes Dev 2001, 15:188, **Figure 1**.

The interference effect can be long lasting and may be detectable after many cell divisions. Moreover, RNAi exhibits sequence specificity. Kisielow, M. *et al.*

(2002) *Isoform-specific knockdown and expression of adaptor protein ShcA using small interfering RNA,* J. of Biochemistry 363: 1-5. Thus, the RNAi machinery can specifically knock down one type of transcript, while not affecting closely related mRNA. These properties make siRNA a potentially valuable tool for inhibiting gene expression and studying gene function and drug target validation. Moreover, siRNAs are potentially useful as therapeutic agents against: (1) diseases that are caused by over-expression or misexpression of genes; and (2) diseases brought about by expression of genes that contain mutations.

Successful siRNA-dependent gene silencing depends on a number of factors. One of the most contentious issues in RNAi is the question of the necessity of siRNA design, *i.e.*, considering the sequence of the siRNA used. Early work in *C. elegans* and plants circumvented the issue of design by introducing long dsRNA (see, for instance, Fire, A. *et al.* (1998) *Nature* 391:806-811). In this primitive organism, long dsRNA molecules are cleaved into siRNA by Dicer, thus generating a diverse population of duplexes that can potentially cover the entire transcript. While some fraction of these molecules are non-functional (*i.e.* induce little or no silencing) one or more have the potential to be highly functional, thereby silencing the gene of interest and alleviating the need for siRNA design. Unfortunately, due to the interferon response, this same approach is unavailable for mammalian systems. While this effect can be circumvented by bypassing the Dicer cleavage step and directly introducing siRNA, this tactic carries with it the risk that the chosen siRNA sequence may be non-functional or semi-functional.

A number of researches have expressed the view that siRNA design is not a crucial element of RNAi. On the other hand, others in the field have begun to explore the possibility that RNAi can be made more efficient by paying attention to the design of the siRNA. Unfortunately, none of the reported methods have provided a satisfactory scheme for reliably selecting siRNA with acceptable levels of functionality. Accordingly, there is a need to develop rational criteria by which to select siRNA with an acceptable level of functionality, and to identify siRNA that have this improved level of functionality, as well as to identify siRNAs that are hyperfunctional.

Summary of the Invention

The present invention is directed to increasing the efficiency of RNAi, particularly in mammalian systems. Accordingly, the present invention provides kits, siRNAs and methods for increasing siRNA efficacy.

According to one embodiment, the present invention provides a kit for gene silencing, wherein said kit is comprised of a pool of at least two siRNA duplexes, each of which is comprised of a sequence that is complementary to a portion of the sequence of one or more target messenger RNA.

According to a second embodiment, the present invention provides a method for optimizing RNA interference by using one or more siRNAs that are optimized according to a formula (or algorithm) selected from:

Formula I

Relative functionality of siRNA= -(GC/3) +($AU_{15\text{-}19}$) –($Tm_{20°C}$)*3 –(G_{13})*3 –(C_{19}) +(A_{19})*2 +(A_3) +(U_{10})+(A_{14}) –(U_5) –(A_{11})

Formula II

Relative functionality of siRNA= -(GC/3) –($AU_{15\text{-}19}$)*3 –(G_{13})*3 –(C_{19}) +(A_{19})*2 +(A_3)

Formula III

Relative functionality of siRNA= -(GC/3) +($AU_{15\text{-}19}$) –($Tm_{20°C}$)*3

Formula IV

Relative functionality of siRNA=

-GC/2+($AU_{15\text{-}19}$)/2-($Tm_{20°C}$)*2 –(G_{13})*3 –(C_{19}) +(A_{19})*2 +(A_3) +(U_{10})+(A_{14}) –(U_5) – (A_{11})

Formula V

Relative functionality of siRNA=-(G_{13})*3 –(C_{19}) +(A_{19})*2 +(A_3) + (U_{10})+(A_{14}) –(U_5) –(A_{11})

Formula VI

Relative functionality of siRNA=-(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3)

Formula VII

Relative functionality of siRNA=-(GC/2) +(AU_{15-19})/2 -($Tm_{20°C}$)*1 -(G_{13})*3 -(C_{19}) +(A_{19})*3 +(A_3)*3 +(U_{10})/2+(A_{14})/2 -(U_5)/2 -(A_{11})/2

wherein in Formulas I – VII:

$Tm_{20°C}$=1 if the Tm is greater than 20°C;

A_{19} = 1 if A is the base at position 19 on the sense strand, otherwise its value is 0;

AU_{15-19} = 0 – 5 depending on the number of A or U bases on the sense strand at positions 15 –19;

G_{13}= 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;

C_{19} = 1 if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC= the number of G and C bases in the entire sense strand;

A_3= 1 if A is the base at position 3 on the sense strand, otherwise its value is 0;

A_{11}= 1 if A is the base at position 11 on the sense strand, otherwise its value is 0;

A_{14}= 1 if A is the base at position 14 on the sense strand, otherwise its value is 0;

U_{10}= 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;

U_5= 1 if U is the base at position 5 on the sense strand, otherwise its value is 0;

or

Formula VIII Relative functionality of siRNA =

(-14)*G_{13}-13*A_1-12*U_7-11*U_2-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19} +7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+

$11*C_9+15*G_1+18*A_3+19*U_{10}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$; and

Formula IX Relative functionality of siRNA =

$(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$

wherein

$A_1 = 1$ if A is the base at position 1 of the sense strand, otherwise its value is 0;

$A_2 = 1$ if A is the base at position 2 of the sense strand, otherwise its value is 0;

$A_3 = 1$ if A is the base at position 3 of the sense strand, otherwise its value is 0;

$A_4 = 1$ if A is the base at position 4 of the sense strand, otherwise its value is 0;

$A_5 = 1$ if A is the base at position 5 of the sense strand, otherwise its value is 0;

$A_6 = 1$ if A is the base at position 6 of the sense strand, otherwise its value is 0;

$A_7 = 1$ if A is the base at position 7 of the sense strand, otherwise its value is 0;

$A_{10} = 1$ if A is the base at position 10 of the sense strand, otherwise its value is 0;

$A_{11} = 1$ if A is the base at position 11 of the sense strand, otherwise its value is 0;

$A_{13} = 1$ if A is the base at position 13 of the sense strand, otherwise its value is 0;

$A_{19} = 1$ if A is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$C_3 = 1$ if C is the base at position 3 of the sense strand, otherwise its value is 0;

$C_4 = 1$ if C is the base at position 4 of the sense strand, otherwise its value is 0;

$C_5 = 1$ if C is the base at position 5 of the sense strand, otherwise its value is 0;

$C_6 = 1$ if C is the base at position 6 of the sense strand, otherwise its value is 0;

$C_7 = 1$ if C is the base at position 7 of the sense strand, otherwise its value is 0;

$C_9 = 1$ if C is the base at position 9 of the sense strand, otherwise its value is 0;

$C_{17} = 1$ if C is the base at position 17 of the sense strand, otherwise its value is 0;

$C_{18} = 1$ if C is the base at position 18 of the sense strand, otherwise its value is 0;

$C_{19} = 1$ if C is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$G_1 = 1$ if G is the base at position 1 on the sense strand, otherwise its value is 0;

$G_2 = 1$ if G is the base at position 2 of the sense strand, otherwise its value is 0;

$G_8 = 1$ if G is the base at position 8 on the sense strand, otherwise its value is 0;

$G_{10} = 1$ if G is the base at position 10 on the sense strand, otherwise its value is 0;

$G_{13} = 1$ if G is the base at position 13 on the sense strand, otherwise its value is 0;

$G_{19} = 1$ if G is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$U_1 = 1$ if U is the base at position 1 on the sense strand, otherwise its value is 0;

$U_2 = 1$ if U is the base at position 2 on the sense strand, otherwise its value is 0;

$U_3 = 1$ if U is the base at position 3 on the sense strand, otherwise its value is 0;

$U_4 = 1$ if U is the base at position 4 on the sense strand, otherwise its value is 0;

$U_7 = 1$ if U is the base at position 7 on the sense strand, otherwise its value is 0;

$U_9 = 1$ if U is the base at position 9 on the sense strand, otherwise its value is 0;

$U_{10} = 1$ if U is the base at position 10 on the sense strand, otherwise its value is 0;

$U_{15} = 1$ if U is the base at position 15 on the sense strand, otherwise its value is 0;

$U_{16} = 1$ if U is the base at position 16 on the sense strand, otherwise its value is 0;

$U_{17} = 1$ if U is the base at position 17 on the sense strand, otherwise its value is 0;

$U_{18} = 1$ if U is the base at position 18 on the sense strand, otherwise its value is 0;

GC_{15-19} = the number of G and C bases within positions 15 – 19 of the sense strand or within positions 15 –18 if the sense strand is only 18 base pairs in length;

GC_{total} = the number of G and C bases in the sense strand;

Tm = 100 if the targeting site contains an inverted repeat longer than 4 base pairs, otherwise its value is 0; and

X = the number of times that the same nucleotide repeats four or more times in a row.

According to a third embodiment, the present invention is directed to a kit comprised of at least one siRNA that contains a sequence that is optimized according to one of the formulas above. Preferably the kit contains at least two optimized siRNA, each of which comprises a duplex, wherein one strand of each duplex comprises at least eighteen contiguous bases that are complementary to a region of a target messenger RNA. For mammalian systems, the siRNA preferably comprises between 18 and 30 nucleotide base pairs.

The ability to use the above algorithms, which are not sequence or species specific, allows for the cost-effective selection of optimized siRNAs for specific target sequences. Accordingly, there will be both greater efficiency and reliability in the use of siRNA technologies.

According to a fourth embodiment, the present invention provides a method for developing an siRNA algorithm for selecting functional and hyperfunctional siRNAs for a given sequence. The method comprises:

(a) selecting a set of siRNAs;

(b) measuring the gene silencing ability of each siRNA from said set;

(c) determining the relative functionality of each siRNA;

(d) determining the amount of improved functionality by the presence or absence of at least one variable selected from the group consisting of the total GC content, melting temperature of the siRNA, GC content at positions 15 –19, the presence or absence of a particular nucleotide at a particular position and the number of times that the same nucleotide repeats within a given sequence; and

(e) developing an algorithm using the information of step (d).

According to this embodiment, preferably the set of siRNAs comprises at least 90 siRNAs from at least one gene, more preferably at least 180 siRNAs from at least two different genes, and most preferably at least 270 and 360 siRNAs from at least three and four different genes, respectively. Additionally, in step (d) the determination is made with preferably at least two, more preferably at least three, even more preferably at least four, and most preferably all of the variables. The resulting algorithm is not target sequence specific.

In a fifth embodiment, the present invention provides rationally designed siRNAs identified using the formulas above.

In a sixth embodiment, the present invention is directed to hyperfunctional siRNA.

For a better understanding of the present invention together with other and further advantages and embodiments, reference is made to the following description taken in conjunction with the examples, the scope of which is set forth in the appended claims.

Brief Description of the Figures

Figure 1 shows a model for siRNA-RISC interactions. RISC has the ability to interact with either end of the siRNA or miRNA molecule. Following binding, the duplex is unwound, and the relevant target is identified, cleaved, and released.

Figure 2 is a representation of the functionality of two hundred and seventy siRNA duplexes that were generated to target human cyclophilin, human diazepam-binding inhibitor (DB), and firefly luciferase.

Figure 3a is a representation of the silencing effect of 30 siRNAs in three different cells lines, HEK293, DU145, and Hela. **Figure 3b** shows the frequency of different functional groups (>95% silencing (black), >80% silencing (gray), >50% silencing (dark gray), and <50% silencing (white)) based on GC content. In cases where a given bar is absent from a particular GC percentage, no siRNA were identified for that particular group. **Figure 3c** shows the frequency of different functional groups based on melting temperature (Tm). Again, each group has four different divisions: >95% (black), >80% (gray), >50% (dark gray), and <50% (white) silencing.

Figure 4 is a representation of a statistical analysis that revealed correlations between silencing and five sequence-related properties of siRNA: (A) an A at position 19 of the sense strand, (B) an A at position 3 of the sense strand, (C) a U at position 10 of the sense strand, (D) a base other than G at position 13 of the sense strand, and (E) a base other than C at position 19 of the sense strand. All variables were correlated with siRNA silencing of firefly luciferase and human cyclophilin. SiRNAs satisfying the criterion are grouped on the left (Selected) while those that do not, are grouped on the right (Eliminated). Y-axis is "% Silencing of Control." Each position on the X-axis represents a unique siRNA.

Figures 5 A and **5 B** are representations of firefly luciferase and cyclophilin siRNA panels sorted according to functionality and predicted values using Formula VIII. The siRNA found within the circle represent those that have Formula VIII values (SMARTscores™) above zero. SiRNA outside the indicated area have calculated Formula VIII values that are below zero. Y-axis is "Expression (% Control)." Each position on the X-axis represents a unique siRNA.

Figure 6A is a representation of the average internal stability profile (AISP) derived from 270 siRNAs taken from three separate genes (cyclophilin B, DBI and firefly luciferase). Graphs represent AISP values of highly functional, functional, and non-functional siRNA. **Figure 6B** is a comparison between the AISP of naturally derived GFP siRNA (filled squares) and the AISP of siRNA from cyclophilin B, DBI, and luciferase having >90% silencing properties (no fill) for the antisense strand. "DG" is the symbol for ΔG, free energy.

Figure 7 is a histogram showing the differences in duplex functionality upon introduction of basepair mismatches. The X-axis shows the mismatch introduced inot the siRNA and the position it is introduced (*e.g.*, 8C->A reveals that position 8 (which normally has a C) has been changed to an A). The Y-axis is "% Silencing (Normalized to Control)."

Figure 8a is histogram that shows the effects of 5'sense and antisense strand modification with 2'-O-methylation on functionality. **Figure 8b** is an expression profile showing a comparison of sense strand off-target effects for IGF1R-3 and 2'-O-methyl IGF1R-3. Sense strand off-targets (lower white box) are not induced when the 5' end of the sense strand is modified with 2'-O-methyl groups (top white box).

Figure 9 shows a graph of SMARTscores™ versus RNAi silencing values for more than 360 siRNA directed against 30 different genes. SiRNA to the right of the vertical bar represent those siRNA that have desirable SMARTscores™.

Figures 10A – E compare the RNAi of five different genes (SEAP, DBI, PLK, Firefly Luciferase, and Renila Luciferase) by varying numbers of randomly selected

siRNA and four rationally designed (SMART-selected) siRNA chosen using the algorithm described in Formula VIII. In addition, RNAi induced by a pool of the four SMART-selected siRNA is reported at two different concentrations (100 and 400nM). **10F** is a comparison between a pool of randomly selected EGFR siRNA (Pool 1) and a pool of SMART selected EGFR siRNA (Pool 2). Pool 1, S1—S4 and Pool 2 S1—S4 represent the individual members that made up each respective pool. Note that numbers for random siRNAs represent the position of the 5' end of the sense strand of the duplex. The Y-axis represents the % expression of the control(s). The X-axis is the percent expression of the control.

Figure 11 shows the Western blot results from cells treated with siRNA directed against twelve different genes involved in the clathrin-dependent endocytosis pathway (CHC, DynII, CALM, CLCa, CLCb, Eps15, Eps15R, Rab5a, Rab5b, Rab5c, β2 subunit of AP-2 and EEA.1). SiRNA were selected using Formula VIII. "Pool" represents a mixture of duplexes 1-4. Total concentration of each siRNA in the pool is 25 nM. Total concentration = 4 x 25 = 100 nM.

Figure 12 is a representation of the gene silencing capabilities of rationally-selected siRNA directed against ten different genes (human and mouse cyclophilin, C-myc, human lamin A/C, QB (ubiquinol-cytochrome c reductase core protein I), MEK1 and MEK2, ATE1 (arginyl-tRNA protein transferase), GAPDH, and Eg5). The Y-axis is the percent expression of the control. Numbers 1, 2, 3 and 4 represent individual rationally selected siRNA. "Pool" represents a mixture of the four individual siRNA.

Figure 13 is the sequence of the top ten Bcl2 siRNAs as determined by Formula VIII. Sequences are listed 5' to 3'.

Figure 14 is the knockdown by the top ten Bcl2 siRNAs at 100nM concentrations. The Y-axis represents the amount of expression relative to the non-specific (ns) and transfection mixture control.

Figure 15 represents a functional walk where siRNA beginning on every other base pair of a region of the luciferase gene are tested for the ability to silence the luciferase

gene. The Y-axis represents the percent expression relative to a control. The X-axis represents the position of each individual siRNA.

Figure 16 is a histogram demonstrating the inhibition of target gene expression by pools of 2 and 3 siRNAs duplexes taken from the walk described in Figure 15. The Y-axis represents the percent expression relative to control. The X-axis represents the position of the first siRNA in paired pools, or trios of siRNA. For instance, the first paired pool contains siRNA 1 and 3. The second paired pool contains siRNA 3 and 5. Pool 3 (of paired pools) contains siRNA 5 and 7, and so on.

Figure 17 is a histogram demonstrating the inhibition of target gene expression by pools of 4 and 5 siRNA duplexes. The Y-axis represents the percent expression relative to a control. The X-axis represents the positoin of the first siRNA in each pool.

Figure 18 is a histogram demonstrating the inhibition of target gene expression by siRNAs that are ten and twenty basepairs apart. The Y-axis represents the percent expression relative to a control. The X-axis represents the position of the first siRNA in each pool.

Figure 19 shows that pools of siRNAs (dark gray bar) work as well (or better) than the best siRNA in the pool (light gray bar). The Y-axis represents the percent expression relative to a control. The-X axis represents the position of the first siRNA in each pool.

Figure 20 shows that the combination of several semifunctional siRNAs (dark gray) result in a significant improvement of gene expression inhibition over individual (semi-functional; light gray) siRNA. The Y-axis represents the percent expression relative to a control.

Figure 21 shows both pools (Library, Lib) and individual siRNAs in inhibition of gene expression of Beta-Galactosidase, Renilla Luciferase and SEAP (alkaline phosphatase). Numbers on the X-axis indicate the position of the 5'-most nucleotide of the sense strand of the duplex. The Y-axis represents the percent expression of

each gene relative to a control. Libraries contain siRNAs that begin at the following nucleotides: Seap: Lib 1: 206, 766, 812,923, Lib 2: 1117, 1280, 1300, 1487, Lib 3: 206, 766, 812, 923, 1117, 1280, 1300,1487, Lib 4: 206, 812, 1117, 1300, Lib 5: 766, 923, 1280, 1487, Lib 6: 206, 1487; Bgal: Lib 1: 979, 1339, 2029, 2590, Lib 2: 1087,1783,2399,3257, Lib 3: 979, 1783, 2590, 3257, Lib 4: 979, 1087, 1339, 1783, 2029, 2399,2590,3257, Lib 5: 979, 1087, 1339, 1783, Lib 6: 2029,2399,2590,3257; Renilla: Lib 1: 174,300,432,568, Lib 2: 592, 633, 729,867, Lib 3: 174, 300, 432, 568, 592, 633,729,867, Lib 4: 174, 432, 592, 729, Lib 5: 300,568,633,867, Lib 6: 592,568.

Figure 22 showS the results of an EGFR and TfnR internalization assay when single gene knockdowns are performed. The Y-axis represents percent internalization relative to control.

Figure 23 shows the results of an EGFR and TfnR internalization assay when multiple genes are knocked down (*e.g.* Rab5a, b, c). The Y-axis represents the percent internalization relative to control.

Figure 24 shows the simultaneous knockdown of four different genes. SiRNAs directed against G6PD, GAPDH, PLK, and UBQ were simultaneously introduced into cells. Twenty-four hours later, cultures were harvested and assayed for mRNA target levels for each of the four genes. A comparison is made between cells transfected with individual siRNAs vs. a pool of siRNAs directed against all four genes.

Figure 25 shows the functionality of ten siRNAs at 0.3nM concentrations.

Detailed Description

Definitions

Unless stated otherwise, the following terms and phrases have the meanings provided below:

siRNA

The term "siRNA" refers to small inhibitory RNA duplexes that induce the RNA interference (RNAi) pathway. These molecules can vary in length (generally between 18-30 basepairS) and contain varying degrees of complementarity to their target mRNA in the antisense strand. Some, but not all, siRNA have unpaired overhanging bases on the 5' or 3' end of the sense strand and/or the antisense strand. The term "siRNA" includes duplexes of two separate strands, as well as single strands that can form hairpin structures comprising a duplex region.

SiRNA may be divided into five (5) groups (non-functional, semi-functional, functional, highly functional, and hyper-functional) based on the level or degree of silencing that they induce in cultured cell lines. As used herein, these definitions are based on a set of conditions where the siRNA is transfected into said cell line at a concentration of 100nM and the level of silencing is tested at a time of roughly 24 hours after transfection, and not exceeding 72 hours after transfection. In this context, "non-functional siRNA" are defined as those siRNA that induce less than 50% (<50%) target silencing. "Semi-functional siRNA" induce 50-79% target silencing. "Functional siRNA" are molecules that induce 80-95% gene silencing. "Highly-functional siRNA" are molecules that induce greater than 95% gene silencing. "Hyperfunctional siRNA" are a special class of molecules: For purposes of this document, hyperfunctional siRNA are defined as those molecules that: (1) induce greater than 95% silencing of a specific target when they are transfected at subnanomolar concentrations (*i.e.*, less than one nanomolar); and/or (2) induce functional (or better) levels of silencing for greater than 96 hours. These relative functionalities (though not intended to be absolutes) may be used to compare siRNAs to a particular target for applications such as functional genomics, target identification and therapeutics.

miRNA

The term "miRNA" refers to microRNA.

Gene silencing

The phrase "gene silencing" refers to a process by which the expression of a specific gene product is lessened or attenuated. Gene silencing can take place by a

variety of pathways. Unless specified otherwise, as used herin, gene silencing refers to decreases in gene product expression that results from RNA interference (RNAi), a defined, though partially characterized pathway whereby small inhibitory RNA (siRNA) act in concert with host proteins (*e.g.* the RNA induced silencing complex, RISC) to degrade messenger RNA (mRNA) in a sequence-dependent fashion. The level of gene silencing can be measured by a variety of means, including, but not limited to, measurement of transcript levels by Northern Blot Analysis, B-DNA techniques, transcription-sensitive reporter constructs, expression profiling (*e.g.* DNA chips), and related technologies. Alternatively, the level of silencing can be measured by assessing the level of the protein encoded by a specific gene. This can be accomplished by performing a number of studies including Western Analysis, measuring the levels of expression of a reporter protein that has *e.g.* fluorescent properties (*e.g.* GFP) or enzymatic activity (*e.g.* alkaline phosphatases), or several other procedures.

Transfection

The term "transfection" refers to a process by which agents are introduced into a cell. The list of agents that can be transfected is large and includes, but is not limited to, siRNA, sense and/or anti-sense sequences, DNA encoding one or more genes and organized into an expression plasmid, proteins, protein fragments, and more. There are multiple methods for transfecting agents into a cell including, but not limited to, electroporation, calcium phosphate-based transfections, DEAE-dextran-based transfections, lipid-based transfections, molecular conjugate-based transfections (*e.g.* polylysine-DNA conjugates), microinjection and others.

Target

The term "target" is used in a variety of different forms throughout this document and is defined by the context in which it is used. "Target mRNA" refers to a messenger RNA to which a given siRNA can be directed against. "Target sequence" and "target site" refer to a sequence within the mRNA to which the sense strand of an siRNA shows varying degrees of homology and the antisense strand exhibits varying degrees of complementarity. The term "siRNA target" can refer to the gene, mRNA, or protein against which an siRNA is directed. Similarly "target silencing" can refer to the state of a gene, or the corresponding mRNA or protein.

Off-target silencing and Off-target interference

The phrases "off-target silencing" and "off-target interference" are defined as degradation of mRNA other than the intended target mRNA due to overlapping and/or partial homology with secondary mRNA messages.

SMARTscore™

The term "SMARTscore™" refers to a number determined by applying any of the Formulas I - Formula IX to a given siRNA sequence. The term "SMART-selected" or "rationally selected" or "rational selection" refers to siRNA that have been selected on the basis of their SMARTscores™.

Complementary

The term "complementary" refers to the ability of polynucleotides to form base pairs with one another. Base pairs are typically formed by hydrogen bonds between nucleotide units in antiparallel polynucleotide strands. Complementary polynucleotide strands can base pair in the Watson-Crick manner (*e.g.*, A to T, A to U, C to G), or in any other manner that allows for the formation of duplexes. As persons skilled in the art are aware, when using RNA as opposed to DNA, uracil rather than thymine is the base that is considered to be complementary to adenosine. However, when a U is denoted in the context of the present invention, the ability to substitute a T is implied, unless otherwise stated.

Perfect complementarity or 100% complementarity refers to the situation in which each nucleotide unit of one polynucleotide strand can hydrogen bond with a nucleotide unit of a second polynucleotide strand. Less than perfect complementarity refers to the situation in which some, but not all, nucleotide units of two strands can hydrogen bond with each other. For example, for two 20-mers, if only two base pairs on each strand can hydrogen bond with each other, the polynucleotide strands exhibit 10% complementarity. In the same example, if 18 base pairs on each strand can hydrogen bond with each other, the polynucleotide strands exhibit 90% complementarity. "Substantial complementarity" refers to polynucleotide strands exhibiting 79% or greater complementarity, excluding regions of the polynucleotide strands, such as overhangs, that are selected so as to be noncomplementary.

("Substantial similarity" refers to polynucleotide strands exhibiting 79% or greater similarity, excluding regions of the polynucleotide strands, such as overhangs, that are selected so as not to be similar.) Thus, for example, two polynucleotides of 29 nucleotide units each, wherein each comprises a di-dT at the 3' terminus such that the duplex region spans 27 bases, and wherein 26 of the 27 bases of the duplex region on each strand are complementary, are substantially complementary since they are 96.3% complementary when excluding the di-dT overhangs.

Deoxynucleotide

The term "deoxynucleotide" refers to a nucleotide or polynucleotide lacking a hydroxyl group (OH group) at the 2' and/or 3' position of a sugar moiety. Instead, it has a hydrogen bonded to the 2' and/or 3' carbon. Within an RNA molecule that comprises one or more deoxynucleotides, "deoxynucleotide" refers to the lack of an OH group at the 2' position of the sugar moiety, having instead a hydrogen bonded directly to the 2' carbon.

Deoxyribonucleotide

The terms "deoxyribonucleotide" and "DNA" refer to a nucleotide or polynucleotide comprising at least one sugar moiety that has an H, rather than an OH, at its 2' and/or 3'position.

Substantially Similar

The phrase "substantially similar" refers to a similarity of at least 90% with respect to the identity of the bases of the sequence.

Duplex Region

The phrase "duplex region" refers to the region in two complementary or substantially complementary polynucleotides that form base pairs with one another, either by Watson-Crick base pairing or any other manner that allows for a stabilized duplex between polynucleotide strands that are complementary or substantially complementary. For example, a polynucleotide strand having 21 nucleotide units can base pair with another polynucleotide of 21 nucleotide units, yet only 19 bases on each strand are complementary or substantially complementary, such that the "duplex region" has 19 base pairs. The remaining bases may, for example, exist as 5' and 3'

overhangs. Further, within the duplex region, 100% complementarity is not required; substantial complementarity is allowable within a duplex region. Substantial complementarity refers to 79% or greater complementarity. For example, a mismatch in a duplex region consisting of 19 base pairs results in 94.7% complementarity, rendering the duplex region substantially complementary.

Nucleotide

The term "nucleotide" refers to a ribonucleotide or a deoxyribonucleotide or modified form thereof, as well as an analog thereof. Nucleotides include species that comprise purines, *e.g.*, adenine, hypoxanthine, guanine, and their derivatives and analogs, as well as pyrimidines, *e.g.*, cytosine, uracil, thymine, and their derivatives and analogs.

Nucleotide analogs include nucleotides having modifications in the chemical structure of the base, sugar and/or phosphate, including, but not limited to, 5-position pyrimidine modifications, 8-position purine modifications, modifications at cytosine exocyclic amines, and substitution of 5-bromo-uracil; and 2'-position sugar modifications, including but not limited to, sugar-modified ribonucleotides in which the 2'-OH is replaced by a group such as an H, OR, R, halo, SH, SR, NH_2, NHR, NR_2, or CN, wherein R is an alkyl moiety. Nucleotide analogs are also meant to include nucleotides with bases such as inosine, queuosine, xanthine, sugars such as 2'-methyl ribose, non-natural phosphodiester linkages such as methylphosphonates, phosphorothioates and peptides.

Modified bases refer to nucleotide bases such as, for example, adenine, guanine, cytosine, thymine, uracil, xanthine, inosine, and queuosine that have been modified by the replacement or addition of one or more atoms or groups. Some examples of types of modifications that can comprise nucleotides that are modified with respect to the base moieties include but are not limited to, alkylated, halogenated, thiolated, aminated, amidated, or acetylated bases, individually or in combination. More specific examples include, for example, 5-propynyluridine, 5-propynylcytidine, 6-methyladenine, 6-methylguanine, N,N,-dimethyladenine, 2-propyladenine, 2-propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5-(2-

amino)propyl uridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 1-methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2-methylguanosine, 7-methylguanosine, 2,2-dimethylguanosine, 5-methylaminoethyluridine, 5-methyloxyuridine, deazanucleotides such as 7-deaza-adenosine, 6-azouridine, 6-azocytidine, 6-azothymidine, 5-methyl-2-thiouridine, other thio bases such as 2-thiouridine and 4-thiouridine and 2-thiocytidine, dihydrouridine, pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O- and N-alkylated purines and pyrimidines such as N6-methyladenosine, 5-methylcarbonylmethyluridine, uridine 5-oxyacetic acid, pyridine-4-one, pyridine-2-one, phenyl and modified phenyl groups such as aminophenol or 2,4,6-trimethoxy benzene, modified cytosines that act as G-clamp nucleotides, 8-substituted adenines and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl nucleotides, carboxyalkylaminoalkyl nucleotides, and alkylcarbonylalkylated nucleotides. Modified nucleotides also include those nucleotides that are modified with respect to the sugar moiety, as well as nucleotides having sugars or analogs thereof that are not ribosyl. For example, the sugar moieties may be, or be based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4'-thioribose, and other sugars, heterocycles, or carbocycles.

The term nucleotide is also meant to include what are known in the art as universal bases. By way of example, universal bases include but are not limited to 3-nitropyrrole, 5-nitroindole, or nebularine. The term "nucleotide" is also meant to include the N3' to P5' phosphoramidate, resulting from the substitution of a ribosyl 3' oxygen with an amine group.

Further, the term nucleotide also includes those species that have a detectable label, such as for example a radioactive or fluorescent moiety, or mass label attached to the nucleotide.

Polynucleotide

The term "polynucleotide" refers to polymers of nucleotides, and includes but is not limited to DNA, RNA, DNA/RNA hybrids including polynucleotide chains of regularly and/or irregularly alternating deoxyribosyl moieties and ribosyl moieties (*i.e.*, wherein alternate nucleotide units have an –OH, then and –H, then an –OH, then

an –H, and so on at the 2' position of a sugar moiety), and modifications of these kinds of polynucleotides, wherein the attachment of various entities or moieties to the nucleotide units at any position are included.

Polyribonucleotide

The term "polyribonucleotide" refers to a polynucleotide comprising two or more modified or unmodified ribonucleotides and/or their analogs. The term "polyribonucleotide" is used interchangeably with the term "oligoribonucleotide."

Ribonucleotide and ribonucleic acid

The term "ribonucleotide" and the phrase "ribonucleic acid" (RNA), refer to a modified or unmodified nucleotide or polynucleotide comprising at least one ribonucleotide unit. A ribonucleotide unit comprises an hydroxyl group attached to the 2' position of a ribosyl moiety that has a nitrogenous base attached in N-glycosidic linkage at the 1' position of a ribosyl moiety, and a moiety that either allows for linkage to another nucleotide or precludes linkage.

Detailed Description of the Invention

The present invention is directed to improving the efficiency of gene silencing by siRNA. Through the inclusion of multiple siRNA sequences that are targeted to a particular gene and/or selecting an siRNA sequence based on certain defined criteria, improved efficiency may be achieved.

The present invention will now be described in connection with preferred embodiments. These embodiments are presented in order to aid in an understanding of the present invention and are not intended, and should not be construed, to limit the invention in any way. All alternatives, modifications and equivalents that may become apparent to those of ordinary skill upon reading this disclosure are included within the spirit and scope of the present invention.

Furthermore, this disclosure is not a primer on RNA interference. Basic concepts known to persons skilled in the art have not been set forth in detail.

Optimizing siRNA

According to one embodiment, the present invention provides a method for improving the effectiveness of gene silencing for use to silence a particular gene through the selection of an optimal siRNA. An siRNA selected according to this method may be used individually, or in conjunction with the first embodiment, *i.e.*, with one or more other siRNAs, each of which may or may not be selected by this criteria in order to maximize their efficiency.

The degree to which it is possible to select an siRNA for a given mRNA that maximizes these criteria will depend on the sequence of the mRNA itself. However, the selection criteria will be independent of the target sequence. According to this method, an siRNA is selected for a given gene by using a rational design. That said, rational design can be described in a variety of ways. Rational design is, in simplest terms, the application of a proven set of criteria that enhance the probability of identifying a functional or hyperfunctional siRNA. In one method, rationally designed siRNA can be identified by maximizing one or more of the following criteria:

1. A low GC content, preferably between about 30 –52%.
2. At least 2, preferably at least 3 A or U bases at positions 15- 19 of the siRNA on the sense strand.
3. An A base at position 19 of the sense strand.
4. An A base at position 3 of the sense strand.
5. A U base at position 10 of the sense strand.
6. An A base at position 14 of the sense strand.
7. A base other than C at position 19 of the sense strand.
8. A base other than G at position 13 of the sense strand.
9. A Tm, which refers to the character of the internal repeat that results in inter- or intramolecular structures for one strand of the duplex, that is preferably not stable at greater than 50°C, more preferably not stable at greater than 37°C, even more preferably not stable at greater than 30°C and most preferably not stable at greater than 20°C.
10. A base other than U at position 5 of the sense strand.
11. A base other than A at position 11 of the sense strand.

Criteria 5, 6, 10 and 11 are minor criteria, but are nonetheless desirable. Accordingly, preferably an siRNA will satisfy as many of the aforementioned criteria as possible, more preferably at least 1 – 4 and 7-9, and most preferably all of the criteria

With respect to the criteria, GC content, as well as a high number of AU in positions 15-19, may be important for easement of the unwinding of double stranded siRNA duplex. Duplex unwinding has been shown to be crucial for siRNA functionality *in vivo*.

With respect to criterion 9, the internal structure is measured in terms of the melting temperature of the single strand of siRNA, which is the temperature at which 50% of the molecules will become denatured. With respect to criteria 2 – 8 and 10 – 11, the positions refer to sequence positions on the sense strand, which is the strand that is identical to the mRNA.

In one preferred embodiment, at least criteria 1 and 8 are satisfied. In another preferred embodiment, at least criteria 7 and 8 are satisfied. In still another preferred embodiment, at least criteria 1, 8 and 9 are satisfied.

It should be noted that all of the aforementioned criteria regarding sequence position specifics are with respect to the 5'-end of the sense strand. Reference is made to the sense strand, because most databases contain information that describes the information of the mRNA. Because according to the present invention a chain can be from 18 to 30 bases in length, and the aforementioned criteria assumes a chain 19 base pairs in length, it is important to keep the aforementioned criteria applicable to the correct bases.

When there are only 18 bases, the base pair that is not present is the base pair that is located at the 3' of the sense strand. When there are twenty to thirty bases present, then additional bases are added at the 5' end of the sense chain and occupy positions $^{-}$1 to $^{-}$11. Accordingly, with respect to SEQ. ID NO. 0001. NNANANNNNUCNAANNNNA and SEQ. ID NO. 0028.

GUCNNANANNNNUCNAANNNNA, both would have A at position 3, A at position 5, U at position 10, C at position 11, A and position 13, A and position 14 and A at position 19. However, SEQ. ID NO. 0028 would also have C at position –1, U at position –2 and G at position –3.

For a 19 base pair siRNA, an optimal sequence of one of the strands may be represented below, where N is any base, A, C, G, or U:

SEQ. ID NO. 0001. NNANANNNNUCNAANNNNA
SEQ. ID NO. 0002. NNANANNNNUGNAANNNNA
SEQ. ID NO. 0003. NNANANNNNUUNAANNNNA
SEQ. ID NO. 0004. NNANANNNNUCNCANNNNA
SEQ. ID NO. 0005. NNANANNNNUGNCANNNNA
SEQ. ID NO. 0006. NNANANNNNUUNCANNNNA
SEQ. ID NO. 0007. NNANANNNNUCNUANNNNA
SEQ. ID NO. 0008.. NNANANNNNUGNUANNNNA
SEQ. ID NO. 0009. NNANANNNNUUNUANNNNA
SEQ. ID NO. 0010. NNANCNNNNUCNAANNNNA
SEQ. ID NO. 0011. NNANCNNNNUGNAANNNNA
SEQ. ID NO. 0012. NNANCNNNNUUNAANNNNA
SEQ. ID NO. 0013. NNANCNNNNUCNCANNNNA
SEQ. ID NO. 0014. NNANCNNNNUGNCANNNNA
SEQ. ID NO. 0015. NNANCNNNNUUNCANNNNA
SEQ. ID NO. 0016. NANCNNNNUCNUANNNNA
SEQ. ID NO. 0017. NNANCNNNNUGNUANNNNA
SEQ. ID NO. 0018. NNANCNNNNUUNUANNNNA
SEQ. ID NO. 0019. NNANGNNNNUCNAANNNNA
SEQ. ID NO. 0020. NNANGNNNNUGNAANNNNA
SEQ. ID NO. 0021. NNANGNNNNUUNAANNNNA
SEQ. ID NO. 0022. NNANGNNNNUCNCANNNNA
SEQ. ID NO. 0023. NNANGNNNNUGNCANNNNA
SEQ. ID NO. 0024. NNANGNNNNUUNCANNNNA
SEQ. ID NO. 0025. NNANGNNNNUCNUANNNNA
SEQ. ID NO. 0026. NNANGNNNNUGNUANNNNA

SEQ. ID NO. 0027. NNANGNNNNNUNUANNNNA

In one embodiment, the sequence used as an siRNA is selected by choosing the siRNA that score highest according to one of the following seven algorithms that are represented by Formulas I - VII:

Formula I

Relative functionality of siRNA= $-(GC/3) +(AU_{15\text{-}19}) -(Tm_{20°C})*3 -(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3) +(U_{10})+(A_{14}) -(U_5) -(A_{11})$

Formula II

Relative functionality of siRNA= $-(GC/3) -(AU_{15\text{-}19})*3 -(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3)$

Formula III

Relative functionality of siRNA= $-(GC/3) +(AU_{15\text{-}19}) -(Tm_{20°C})*3$

Formula IV

Relative functionality of siRNA=
$-GC/2+(AU_{15\text{-}19})/2-(Tm_{20°C})*2 -(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3) +(U_{10})+(A_{14}) -(U_5) -(A_{11})$

Formula V

Relative functionality of siRNA=$-(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3) + (U_{10})+(A_{14}) -(U_5) -(A_{11})$

Formula VI

Relative functionality of siRNA=$-(G_{13})*3 -(C_{19}) +(A_{19})*2 +(A_3)$

Formula VII

Relative functionality of siRNA=$-(GC/2) +(AU_{15\text{-}19})/2 -(Tm_{20°C})*1 -(G_{13})*3 -(C_{19}) +(A_{19})*3 +(A_3)*3 +(U_{10})/2+(A_{14})/2 -(U_5)/2 -(A_{11})/2$

In Formulas I – VII:

wherein A_{19} = 1 if A is the base at position 19 on the sense strand, otherwise its value is 0,

$AU_{15\text{-}19}$ = 0 – 5 depending on the number of A or U bases on the sense strand at positions 15 –19;

G_{13} = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;

C_{19} = 1 if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC= the number of G and C bases in the entire sense strand;

$Tm_{20°C}$=1 if the Tm is greater than 20°C;

A_3= 1 if A is the base at position 3 on the sense strand, otherwise its value is 0;

U_{10}= 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;

A_{14} = 1 if A is the base at position 14 on the sense strand, otherwise its value is 0;

U_5 = 1 if U is the base at position 5 on the sense strand, otherwise its value is 0; and

A_{11} = 1 if A is the base at position 11 of the sense strand, otherwise its value is 0.

Formulas I –VII provide relative information regarding functionality. When the values for two sequences are compared for a given formula, the relative functionality is ascertained; a higher positive number indicates a greater functionality. For example, in many applications a value of 5 or greater is beneficial.

Additionally, in many applications, more than one of these formulas would provide useful information as to the relative functionality of potential siRNA sequences. However, it is beneficial to have more than one type of formula, because not every formula will be able to help to differentiate among potential siRNA sequences. For example, in particularly high GC mRNAs, formulas that take that parameter into account would not be useful and application of formulas that lack GC elements (*e.g.*, formulas V and VI) might provide greater insights into duplex functionality. Similarly, formula II might by used in situations where hairpin

structures are not observed in duplexes, and formula IV might be applicable for sequences that have higher AU content. Thus, one may consider a particular sequence in light of more than one or even all of these algorithms to obtain the best differentiation among sequences. In some instances, application of a given algorithim may identify an unususally large number of potential siRNA sequences, and in those cases, it may be appropriate to re-analyze that sequence with a second algorithm that is, for instance, more stringent. Alternatively, it is conceivable that analysis of a sequence with a given formula yields no acceptable siRNA sequences (*i.e.* low SMARTscores™). In this instance, it may be appropriate to re-analyze that sequences with a second algorithm that is, for instance, less stringent. In still other instances, analysis of a single sequence with two separate formulas may give rise to conflicting results (*i.e.* one formula generates a set of siRNA with high SMARTscores™ while the other formula identifies a set of siRNA with low SMARTscores™). In these instances, it may be necessary to determine which weighted factor(s) (*e.g.* GC content) are contributing to the discrepancy and assessing the sequence to decide whether these factors should or should not be included. Alternatively, the sequence could be analyzed by a third, fourth, or fifth algorithm to identify a set of rationally designed siRNA.

The above-referenced criteria are particularly advantageous when used in combination with pooling techniques as depicted in Table I:

Table I

Criteria	Functional Probability					
	Oligos			Pools		
	>95%	>80%	<70%	>95%	>80%	<70%
Current	33.0	50.0	23.0	79.5	97.3	0.3
New	50.0	88.5	8.0	93.8	99.98	0.005
(GC)	28.0	58.9	36.0	72.8	97.1	1.6

The term "current" refers to Tuschl's conventional siRNA parameters (Elbashir, S.M. et al. (2002) "Analysis of gene function in somatic mammalian cells using small interfering RNAs" Methods 26: 199-213). "New" refers to the design parameters

described in Formulas I-VII. "GC" refers to criteria that select siRNA solely on the basis of GC content.

As Table I indicates, when more functional siRNA duplexes are chosen, siRNAs that produce <70% silencing drops from 23% to 8% and the number of siRNA duplexes that produce >80% silencing rises from 50% to 88.5%. Further, of the siRNA duplexes with >80% silencing, a larger portion of these siRNAs actually silence >95% of the target expression (the new criteria increases the portion from 33% to 50%). Using this new criteria in pooled siRNAs, shows that, with pooling, the amount of silencing >95% increases from 79.5% to 93.8% and essentially eliminates any siRNA pool from silencing less than 70%.

Table II similarly shows the particularly beneficial results of pooling in combination with the aforementioned criteria. However, Table II, which takes into account each of the aforementioned variables, demonstrates even a greater degree of improvement in functionality.

Table II

	Functional Probability					
	Oligos			Pools		
	Functional	Average	Non-functional	Functional	Average	Non-functional
Random	20	40	50	67	97	3
Criteria 1	52	99	0.1	97	93	0.0040
Criteria 4	89	99	0.1	99	99	0.0000

The terms "functional," "Average," and "Non-functional" refer to siRNA that exhibit >80%, >50%, and <50% functionality, respectively. Criteria 1 and 4 refer to specific criteria described above.

The above-described algorithms may be used with or without a computer program that allows for the inputting of the sequence of the mRNA and automatically outputs the optimal siRNA. The computer program may, for example, be accessible

from a local terminal or personal computer, over an internal network or over the Internet.

In addition to the formulas above, more detailed algorithms may be used for selecting siRNA. Preferably, at least one RNA duplex of between 18 and 30 base pairs is selected such that it is optimized according a formula selected from:

Formula VIII: $(-14)*G_{13}-13*A_1-12*U_7-11*U_2-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19}+7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+11*C_9+15*G_1+18*A_3+19*U_{10}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$; and

Formula IX: $(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$

wherein

$A_1 = 1$ if A is the base at position 1 of the sense strand, otherwise its value is 0;

$A_2 = 1$ if A is the base at position 2 of the sense strand, otherwise its value is 0;

$A_3 = 1$ if A is the base at position 3 of the sense strand, otherwise its value is 0;

$A_4 = 1$ if A is the base at position 4 of the sense strand, otherwise its value is 0;

$A_5 = 1$ if A is the base at position 5 of the sense strand, otherwise its value is 0;

$A_6 = 1$ if A is the base at position 6 of the sense strand, otherwise its value is 0;

$A_7 = 1$ if A is the base at position 7 of the sense strand, otherwise its value is 0;

$A_{10} = 1$ if A is the base at position 10 of the sense strand, otherwise its value is 0;

$A_{11} = 1$ if A is the base at position 11 of the sense strand, otherwise its value is 0;

$A_{13} = 1$ if A is the base at position 13 of the sense strand, otherwise its value is 0;

$A_{19} = 1$ if A is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

C_3 = 1 if C is the base at position 3 of the sense strand, otherwise its value is 0;
C_4 = 1 if C is the base at position 4 of the sense strand, otherwise its value is 0;
C_5 = 1 if C is the base at position 5 of the sense strand, otherwise its value is 0;
C_6 = 1 if C is the base at position 6 of the sense strand, otherwise its value is 0;
C_7 = 1 if C is the base at position 7 of the sense strand, otherwise its value is 0;
C_9 = 1 if C is the base at position 9 of the sense strand, otherwise its value is 0;
C_{17} = 1 if C is the base at position 17 of the sense strand, otherwise its value is 0;
C_{18} = 1 if C is the base at position 18 of the sense strand, otherwise its value is 0;
C_{19} = 1 if C is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

G_1 = 1 if G is the base at position 1 on the sense strand, otherwise its value is 0;
G_2 = 1 if G is the base at position 2 of the sense strand, otherwise its value is 0;
G_8 = 1 if G is the base at position 8 on the sense strand, otherwise its value is 0;
G_{10} = 1 if G is the base at position 10 on the sense strand, otherwise its value is 0;
G_{13} = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;
G_{19} = 1 if G is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

U_1 = 1 if U is the base at position 1 on the sense strand, otherwise its value is 0;
U_2 = 1 if U is the base at position 2 on the sense strand, otherwise its value is 0;
U_3 = 1 if U is the base at position 3 on the sense strand, otherwise its value is 0;
U_4 = 1 if U is the base at position 4 on the sense strand, otherwise its value is 0;
U_7 = 1 if U is the base at position 7 on the sense strand, otherwise its value is 0;
U_9 = 1 if U is the base at position 9 on the sense strand, otherwise its value is 0;
U_{10} = 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;
U_{15} = 1 if U is the base at position 15 on the sense strand, otherwise its value is 0;
U_{16} = 1 if U is the base at position 16 on the sense strand, otherwise its value is 0;
U_{17} = 1 if U is the base at position 17 on the sense strand, otherwise its value is 0;
U_{18} = 1 if U is the base at position 18 on the sense strand, otherwise its value is 0;

GC_{15-19} = the number of G and C bases within positions 15 – 19 of the sense strand, or within positions 15 –18 if the sense strand is only 18 base pairs in length;

GC_{total} = the number of G and C bases in the sense strand;

Tm = 100 if the siRNA oligo has the internal repeat longer then 4 base pairs, otherwise its value is 0; and

X = the number of times that the same nucleotide repeats four or more times in a row.

The above formulas VIII and IX, as well as formulas I – VII, provide methods for selecting siRNA in order to increase the efficiency of gene silencing. A subset of variables of any of the formulas may be used, though when fewer variables are used, the optimization hierarchy becomes less reliable.

With respect to the variables of the above-referenced formulas, a single letter of A or C or G or U followed by a subscript refers to a binary condition. The binary condition is that either the particular base is present at that particular position (wherein the value is "1") or the base is not present (wherein the value is "0"). Because position 19 is optional, *i.e.* there might be only 18 base pairs, when there are only 18 base pairs, any base with a subscript of 19 in the formulas above would have a zero value for that parameter. Before or after each variable is a number followed by *, which indicates that the value of the variable is to be multiplied or weighed by that number.

The numbers preceding the variables A, or G, or C, or U in Formulas VIII and IX (or after the variables in Formula I - VII) were determined by comparing the difference in the frequency of individual bases at different positions in functional siRNA and total siRNA. Specifically, the frequency in which a given base was observed at a particular position in functional groups was compared with the frequency that that same base was observed in the total, randomly selected siRNA set. If the absolute value of the difference between the functional and total values was found to be greater than 6%, that parameter was included in the equation. Thus for instance, if the frequency of finding a "G" at position 13 (G_{13}) is found to be 6% in a given functional group, and the frequency of G_{13} in the total population of siRNAs is 20%, the difference between the two values is 6%-20% = -14%. As the absolute value is greater than six (6), this factor (-14) is included in the equation. Thus in Formula VIII, in cases where the siRNA under study has a G in position 13, the accrued value

is (-14) * (1) = -14. In contrast, when a base other than G is found at position 13, the accrued value is (-14) * (0) = 0.

When developing a means to optimize siRNAs, the inventors observed that a bias toward low internal thermodynamic stability of the duplex at the 5'-antisense (AS) end is characteristic of naturally occurring miRNA precursors. The inventors extended this observation to siRNAs for which functionality had been assessed in tissue culture.

With respect to the parameter GC_{15-19}, a value of 0 – 5 will be ascribed depending on the number of G or C bases at positions 15 to 19. If there are only 18 base pairs, the value is between 0 and 4.

With respect to the criterion GC_{total} content, a number from 0 – 30 will be ascribed, which correlates to the total number of G and C nucleotides on the sense strand, excluding overhangs. Without wishing to be bound by any one theory, it is postulated that the significance of the GC content (as well as AU content at positions 15-19, which is a parameter for formulas III – VII) relates to the easement of the unwinding of a double-stranded siRNA duplex. Duplex unwinding is believed to be crucial for siRNA functionality *in vivo* and overall low internal stability, especially low internal stability of the first unwound base pair is believed to be important to maintain sufficient processivity of RISC complex-induced duplex unwinding. If the duplex has 19 base pairs, those at positions 15-19 on the sense strand will unwind first if the molecule exhibits a sufficiently low internal stability at that position. As persons skilled in the art are aware, RISC is a complex of approximately twelve proteins; Dicer is one, but not the only, helicase within this complex. Accordingly, although the GC parameters are believed to relate to activity with Dicer, they are also important for activity with other RISC proteins.

The value of the parameter Tm is 0 when there are no internal repeats longer than (or equal to) four base pairs present in the siRNA duplex; otherwise the value is 1. Thus for example, if the sequence ACGUACGU, or any other four nucleotide (or more) palindrome exists within the structure, the value will be one (1). Alternatively

if the structure ACGGACG, or any other 3 nucleotide (or less) palindrome exists, the value will be zero (0).

The variable "X" refers to the number of times that the same nucleotide occurs contiguously in a stretch of four or more units. If there are, for example, four contiguous As in one part of the sequence and elsewhere in the sequence four contiguous Cs, X =2. Further, if there are two separate contiguous stretches of four of the same nucleotides or eight or more of the same nucleotides in a row, then X=2. However, X does not increase for five, six or seven contiguous nucleotides.

Again, when applying Formula VIII or Formula IX to a given mRNA, (the "target RNA" or "target molecule"), one may use a computer program to evaluate the criteria for every sequence of 18 – 30 base pairs or only sequences of a fixed length, *e.g.*, 19 base pairs. Preferably the computer program is designed such that it provides a report ranking of all of the potential siRNAs between 18 and 30 base pairs, ranked according to which sequences generate the highest value. A higher value refers to a more efficient siRNA for a particular target gene. The computer program that may be used, may be developed in any computer language that is known to be useful for scoring nucleotide sequences, or it may be developed with the assistance of commercially available product such as Microsoft's product .net. Additionally, rather than run every sequence through one and/or another formula, one may compare a subset of the sequences, which may be desirable if for example only a subset are available. For instance, it may be desirable to first perform a BLAST (Basic Local Alignment Search Tool) search and to identify sequences that have no homology to other targets. Alternatively, it may be desirable to scan the sequence and to identify regions of moderate GC context, then perform relevant calculations using one of the above-described formulas on these regions. These calculations can be done manually or with the aid of a computer.

As with Formulas I – VII, either Formula VIII or Formula IX may be used for a given mRNA target sequence. However, it is possible that according to one or the other formula more than one siRNA will have the same value. Accordingly, it is beneficial to have a second formula by which to differentiate sequences. Formula IX was derived in a similar fashion as Formula VIII, yet used a larger data set and thus

yields sequences with higher statistical correlations to highly functional duplexes. The sequence that has the highest value ascribed to it may be referred to as a "first optimized duplex." The sequence that has the second highest value ascribed to it may be referred to as a "second optimized duplex." Similarly, the sequences that have the third and fourth highest values ascribed to them may be referred to as a third optimized duplex and a fourth optimized duplex, respectively. When more than one sequence has the same value, each of them may, for example, be referred to as first optimized duplex sequences or co-first optimized duplexes.

SiRNA sequences identified using Formula VIII are contained within the enclosed compact disks. The data included on the enclosed compact disks is described more fully below. The sequences identified by Formula VIII that are disclosed in the compacts disks may be used in gene silencing applications.

It should be noted that for Formulas VIII and IX all of the aforementioned criteria are identified as positions on the sense strand when oriented in the 5' to 3' direction as they are identified in connection with Formulas I – VII unless otherwise specified.

Formulas I - IX, may be used to select or to evaluate one, or more than one, siRNA in order to optimize silencing. Preferably, at least two optimized siRNAs that have been selected according to at least one of these formulas are used to silence a gene, more preferably at least three and most preferably at least four. The siRNAs may be used individually or together in a pool or kit. Further, they may be applied to a cell simultaneously or separately. Preferably, the at least two siRNAs are applied simultaneously. Pools are particularly beneficial for many research applications. However, for therapeutics, it may be more desirable to employ a single hyperfunctional siRNA as described elsewhere in this application.

When planning to conduct gene silencing, and it is necessary to choose between two or more siRNAs, one should do so by comparing the relative values when the siRNA are subjected to one of the formulas above. In general a higher scored siRNA should be used.

Useful applications include, but are not limited to, target validation, gene functional analysis, research and drug discovery, gene therapy and therapeutics. Methods for using siRNA in these applications are well known to persons of skill in the art.

Because the ability of siRNA to function is dependent on the sequence of the RNA and not the species into which it is introduced, the present invention is applicable across a broad range of species, including but not limited to all mammalian species, such as humans, dogs, horses, cats, cows, mice, hamsters, chimpanzees and gorillas, as well as other species and organisms such as bacteria, viruses, insects, plants and *C. elegans*.

The present invention is also applicable for use for silencing a broad range of genes, including but not limited to the roughly 45,000 genes of a human genome, and has particular relevance in cases where those genes are associated with diseases such as diabetes, Alzheimer's, cancer, as well as all genes in the genomes of the aforementioned organisms.

The siRNA selected according to the aforementioned criteria or one of the aforementioned algorithms are also, for example, useful in the simultaneous screening and functional analysis of multiple genes and gene families using high throughput strategies, as well as in direct gene suppression or silencing.

Development of the Algorithms

To identify siRNA sequence features that promote functionality and to quantify the importance of certain currently accepted conventional factors—such as G/C content and target site accessibility—the inventors synthesized an siRNA panel consisting of 270 siRNAs targeting three genes, Human Cyclophilin, Firefly Luciferase, and Human DBI. In all three cases, siRNAs were directed against specific regions of each gene. For Human Cyclophilin and Firefly Luciferase, ninety siRNAs were directed against a 199 bp segment of each respective mRNA. For DBI, 90 siRNAs were directed against a smaller, 109 base pair region of the mRNA. The sequences to which the siRNAs were directed are provided below.

It should be noted that in certain sequences, "t" is present. This is because many databases contain information in this manner. However, the t denotes a uracil residue in mRNA and siRNA. Any algorithm will, unless otherwise specified, process a t in a sequence as a u.

Human cyclophilin: 193—390, M60857

SEQ. ID NO. 29:

gttccaaaaacagtggataattttgtggccttagctacaggagagaaaggatttggctacaaaaacagcaaattccatcgtgt
aatcaaggacttcatgatccagggcggagacttcaccaggggagatggcacaggaggaaagagcatctacggtgagcg
cttccccgatgagaacttcaaactgaagcactacgggcctggctggg

Firefly luciferase: 1434—1631, U47298 (pGL3, Promega)

SEQ. ID NO. 30:

tgaacttcccgccgccgttgttgttttggagcacggaaagacgatgacggaaaaagagatcgtggattacgtcgccagtca
agtaacaaccgcgaaaaagttgcgcggaggagttgtgtttgtggacgaagtaccgaaaggtcttaccggaaaactcgacg
caagaaaaatcagagagatcctcataaaggccaagaagg

DBI, NM_020548 (202-310) (every position)

SEQ. ID N0. 0031:

acgggcaaggccaagtgggatgcctggaatgagctgaaaggga cttccaaggaagatgccatgaaagcttacatcaaca
aagtagaagagctaaagaaaaaatacggg

A list of the siRNAs appears in Table III (see Examples Section, Example II)

The set of duplexes was analyzed to identify correlations between siRNA functionality and other biophysical or thermodynamic properties. When the siRNA panel was analyzed in functional and non-functional subgroups, certain nucleotides were much more abundant at certain positions in functional or non-functional groups. More specifically, the frequency of each nucleotide at each position in highly functional siRNA duplexes was compared with that of nonfunctional duplexes in order to assess the preference for or against any given nucleotide at every position. These analyses were used to determine important criteria to be included in the siRNA algorithms (Formulas VIII and IX).

The data set was also analyzed for distinguishing biophysical properties of siRNAs in the functional group, such as optimal percent of GC content, propensity for internal structures and regional thermodynamic stability. Of the presented criteria, several are involved in duplex recognition, RISC activation/duplex unwinding, and target cleavage catalysis.

The original data set that was the source of the statistically derived criteria is shown in **Figure 2**. Additionally, this figure shows that random selection yields siRNA duplexes with unpredictable and widely varying silencing potencies as measured in tissue culture using HEK293 cells. In the figure, duplexes are plotted such that each x-axis tick-mark represents an individual siRNA, with each subsequent siRNA differing in target position by two nucleotides for Human Cyclophilin and Firefly Luciferase, and by one nucleotide for Human DBI. Furthermore, the y-axis denotes the level of target expression remaining after transfection of the duplex into cells and subsequent silencing of the target.

SiRNA identified and optimized in this document work equally well in a wide range of cell types. **Figure 3a** shows the evaluation of thirty siRNAs targeting the DBI gene in three cell lines derived from different tissues. Each DBI siRNA displays very similar functionality in HEK293 (ATCC, CRL-1573, human embryonic kidney), HeLa (ATCC, CCL-2, cervical epithelial adenocarcinoma) and DU145 (HTB-81, prostate) cells as deterimined by the B-DNA assay. Thus, siRNA functionality is determined by the primary sequence of the siRNA and not by the intracellular environment. Additionally, it should be noted that although the present invention provides for a determination of the functionality of siRNA for a given target, the same siRNA may silence more than one gene. For example, the complementary sequence of the silencing siRNA may be present in more than one gene. Accordingly, in these circumstances, it may be desirable not to use the siRNA with highest SMARTscore™. In such circumstances, it may be desirable to use the siRNA with the next highest SMARTscore™.

To determine the relevance of G/C content in siRNA function, the G/C content of each duplex in the panel was calculated and the functional classes of siRNAs ($<$F50, $\geq$ F50, $\geq$ F80, $\geq$ F95 where F refers to the percent gene silencing) were sorted

accordingly. The majority of the highly-functional siRNAs (≥F95) fell within the G/C content range of 36%—52% (**Figure 3B**). Twice as many non-functional (< F50) duplexes fell within the high G/C content groups (>57% GC content) compared to the 36%—52% group. The group with extremely low GC content (26% or less) contained a higher proportion of non-functional siRNAs and no highly-functional siRNAs. The G/C content range of 30%—52% was therefore selected as Criterion I for siRNA functionality, consistent with the observation that a G/C range 30%—70% promotes efficient RNAi targeting. Application of this criterion alone provided only a marginal increase in the probability of selecting functional siRNAs from the panel: selection of F50 and F95 siRNAs was improved by 3.6% and 2.2%, respectively. The siRNA panel presented here permitted a more systematic analysis and quantification of the importance of this criterion than that used previously.

A relative measure of local internal stability is the A/U base pair (bp) content; therefore, the frequency of A/U bp was determined for each of the five terminal positions of the duplex (5' sense (S)/5' antisense (AS)) of all siRNAs in the panel. Duplexes were then categorized by the number of A/U bp in positions 1—5 and 15—19 of the sense strand. The thermodynamic flexibility of the duplex 5'-end (positions 1—5; S) did not appear to correlate appreciably with silencing potency, while that of the 3'-end (positions 15—19; S) correlated with efficient silencing. No duplexes lacking A/U bp in positions 15—19 were functional. The presence of one A/U bp in this region conferred some degree of functionality, but the presence of three or more A/Us was preferable and therefore defined as Criterion II. When applied to the test panel, only a marginal increase in the probability of functional siRNA selection was achieved: a 1.8% and 2.3% increase for F50 and F95 duplexes, respectively (**Table IV**).

The complementary strands of siRNAs that contain internal repeats or palindromes may form internal fold-back structures. These hairpin-like structures exist in equilibrium with the duplexed form effectively reducing the concentration of functional duplexes. The propensity to form internal hairpins and their relative stability can be estimated by predicted melting temperatures. High Tm reflects a tendency to form hairpin structures. Lower Tm values indicate a lesser tendency to form hairpins. When the functional classes of siRNAs were sorted by T_m (**Figure 3c**),

the following trends were identified: duplexes lacking stable internal repeats were the most potent silencers (no F95 duplex with predicted hairpin structure $T_m > 60$ °C). In contrast, about 60% of the duplexes in the groups having internal hairpins with calculated T_m values less than 20 °C were F80. Thus, the stability of internal repeats is inversely proportional to the silencing effect and defines Criterion III (predicted hairpin structure $T_m \leq 20$ °C).

Sequence-based determinants of siRNA functionality

When the siRNA panel was sorted into functional and non-functional groups, the frequency of a specific nucleotide at each position in a functional siRNA duplex was compared with that of a nonfunctional duplex in order to assess the preference for or against a certain nucleotide. **Figure 4** shows the results of these queries and the subsequent resorting of the data set (from Figure 2). The data is separated into two sets: those duplexes that meet the criteria, a specific nucleotide in a certain position - grouped on the left (Selected) and those that do not - grouped on the right (Eliminated). The duplexes are further sorted from most functional to least functional with the y-axis of Figure 4a-e representing the % expression *i.e.* the amount of silencing that is elicited by the duplex (Note: each position on the X-axis represents a different duplex). Statistical analysis revealed correlations between silencing and several sequence-related properties of siRNAs. **Figure 4** and **Table IV** show quantitative analysis for the following five sequence-related properties of siRNA: (A) an A at position 19 of the sense strand; (B) an A at position 3 of the sense strand; (C) a U at position 10 of the sense strand; (D) a base other than G at position 13 of the sense strand; and (E) a base other than C at position 19 of the sense strand.

When the siRNAs in the panel were evaluated for the presence of an A at position 19 of the sense strand, the percentage of non-functional duplexes decreased from 20% to 11.8%, and the percentage of F95 duplexes increased from 21.7% to 29.4% (**Table IV**). Thus, the presence of an A in this position defined Criterion IV.

Another sequence-related property correlated with silencing was the presence of an A in position 3 of the sense strand (**Figure 4b**). Of the siRNAs with A3, 34.4% were F95, compared with 21.7% randomly selected siRNAs. The presence of a U base in position 10 of the sense strand exhibited an even greater impact (**Figure 4c**).

Of the duplexes in this group, 41.7% were F95. These properties became criteria V and VI, respectively.

Two negative sequence-related criteria that were identified also appear on **Figure 4**. The absence of a G at position 13 of the sense strand, conferred a marginal increase in selecting functional duplexes (**Figure 4d**). Similarly, lack of a C at position 19 of the sense strand also correlated with functionality (**Figure 4e**). Thus, among functional duplexes, position 19 was most likely occupied by A, and rarely occupied by C. These rules were defined as criteria VII and VIII, respectively.

Application of each criterion individually provided marginal but statistically significant increases in the probability of selecting a potent siRNA. Although the results were informative, the inventors sought to maximize potency and therefore consider multiple criteria or parameters. Optimization is particularly important when developing therapeutics. Interestingly, the probability of selecting a functional siRNA based on each thermodynamic criteria was 2%—4% higher than random, but 4%—8% higher for the sequence-related determinates. Presumably, these sequence-related increases reflect the complexity of the RNAi mechanism and the multitude of protein-RNA interactions that are involved in RNAi-mediated silencing.

Table IV

Criterion	% Functional		Improvement over Random
I. 30%—52% G/C content	< F50	16.4%	-3.6%
	≥ F50	83.6%	3.6%
	≥ F80	60.4%	4.3%
	≥ F95	23.9%	2.2%
II. At least 3 A/U bases at positions 15—19 of the sense strand	< F50	18.2%	-1.8%
	≥ F50	81.8%	1.8%
	≥ F80	59.7%	3.6%
	≥ F95	24.0%	2.3%
III. Absence of internal repeats, as measured by T_m of secondary structure ≤ 20°C	< F50	16.7%	-3.3%
	≥ F50	83.3%	3.3%
	≥ F80	61.1%	5.0%
	≥ F95	24.6%	2.9%
IV. An A base at position 19 of the sense strand	< F50	11.8%	-8.2%
	≥ F50	88.2%	8.2%
	≥ F80	75.0%	18.9%
	≥ F95	29.4%	7.7%
V. An A base at position 3 of the sense strand	< F50	17.2%	-2.8%
	≥ F50	82.8%	2.8%
	≥ F80	62.5%	6.4%
	≥ F95	34.4%	12.7%
VI. A U base at position 10 of the sense strand	< F50	13.9%	-6.1%
	≥ F50	86.1%	6.1%
	≥ F80	69.4%	13.3%
	≥ F95	41.7%	20%
VII. A base other than C at position 19 of the sense strand	< F50	18.8%	-1.2%
	≥ F50	81.2%	1.2%
	≥ F80	59.7%	3.6%
	≥ F95	24.2%	2.5%
VIII. A base other than G at position 13 of the sense strand	< F50	15.2%	-4.8%
	≥ F50	84.8%	4.8%
	≥ F80	61.4%	5.3%
	≥ F95	26.5%	4.8%

The siRNA selection algorithm

In an effort to improve selection further, all identified criteria, including but not limited to those listed in Table IV were combined into the algorithms embodied in Formula VIII and Formula IX. Each siRNA was then assigned a score (referred to as a SMARTscore™) according to the values derived from the formulas. Duplexes that scored higher than 0 or 20, for Formulas VIII and IX, respectively, effectively selected a set of functional siRNAs and excluded all non-functional siRNAs. Conversely, all duplexes scoring lower than 0 and 20 according to formulas VIII and

IX, respectively, contained some functional siRNAs but included all non-functional siRNAs. A graphical representation of this selection is shown in **Figure 5**.

The methods for obtaining the seven criteria embodied in Table IV are illustrative of the results of the process used to develop the information for Formulas VIII and IX. Thus similar techniques were used to establish the other variables and their multipliers. As described above, basic statistical methods were use to determine the relative values for these multipliers.

To determine the value for "Improvement over Random" the difference in the frequency of a given attribute (*e.g.* GC content, base preference) at a particular position is determined between individual functional groups (*e.g.* <F50) and the total siRNA population studied (*e.g.* 270 siRNA molecules selected randomly). Thus, for instance, in Criterion I (30%-52% GC content) members of the <F50 group were observed to have GC contents between 30-52% in 16.4% of the cases. In contrast, the total group of 270 siRNAs had GC contents in this range, 20% of the time. Thus for this particular attribute, there is a small negative correlation between 30%-52% GC content and this functional group (*i.e.* 16.4%-20% = -3.6%). Similarly, for Criterion VI, (a "U" at position 10 of the sense strand), the >F95 group contained a "U" at this position 41.7% of the time. In contrast, the total group of 270 siRNAs had a "U" at this position 21.7% of the time, thus the improvement over random is calculated to be 20% (or 41.7%-21.7%).

Identifying The Average Internal Stability Profile of Strong siRNA

In order to identify an internal stability profile that is characteristic of strong siRNA, 270 different siRNAs derived from the cyclophilin B, the diazepam binding inhibitor (DBI), and the luciferase gene were individually transfected into HEK293 cells and tested for their ability to induce RNAi of the respective gene. Based on their performance in the *in vivo* assay, the sequences were then subdivided into three groups, (i) >95% silencing; (ii) 80-95% silencing; and (iii) less than 50% silencing. Sequences exhibiting 51-84% silencing were eliminated from further consideration to reduce the difficulties in identifying relevant thermodynamic patterns.

Following the division of siRNA into three groups, a statistical analysis was performed on each member of each group to determine the average internal stability profile (AISP) of the siRNA. To accomplish this the Oligo 5.0 Primer Analysis Software and other related statistical packages (*e.g.* Excel) were exploited to determine the internal stability of pentamers using the nearest neighbor method described by Freier *et al.*, (1986) *Improved free-energy parameters for predictions of RNA duplex stability*, Proc Natl. Acad. Sci. U. S. A. 83(24): 9373-7. Values for each group at each position were then averaged, and the resulting data were graphed on a linear coordinate system with the Y-axis expressing the ΔG (free energy) values in kcal/mole and the X-axis identifying the position of the base relative to the 5' end.

The results of the analysis identified multiple key regions in siRNA molecules that were critical for successful gene silencing. At the 3'-most end of the sense strand (5'antisense), highly functional siRNA (>95% gene silencing, see **Figure 6a**, >F95) have a low internal stability (AISP of position 19 = ~ -7.6kcal/mol). In contrast low-efficiency siRNA (*i.e.* those exhibiting less than 50% silencing, <F50) display a distinctly different profile, having high ΔG values (~ -8.4kcal/mol) for the same position. Moving in a 5' (sense strand) direction, the internal stability of highly efficient siRNA rises (position 12 = ~ -8.3kcal/mole) and then drops again (position 7 = ~ -7.7kcal/mol) before leveling off at a value of approximately –8.1kcal/mol for the 5' terminus. SiRNA with poor silencing capabilities show a distinctly different profile. While the AISP value at position 12 is nearly identical with that of strong siRNAs, the values at positions 7 and 8 rise considerably, peaking at a high of ~ -9.0 kcal/mol. In addition, at the 5' end of the molecule the AISP profile of strong and weak siRNA differ dramatically. Unlike the relatively strong values exhibited by siRNA in the >95% silencing group, siRNAs that exhibit poor silencing activity have weak AISP values (-7.6, -7.5, and -7.5 kcal/mol for positions 1, 2 and 3 respectively).

Overall the profiles of both strong and weak siRNAs form distinct sinusoidal shapes that are roughly 180° out-of-phase with each other. While these thermodynamic descriptions define the archetypal profile of a strong siRNA, it will likely be the case that neither the ΔG values given for key positions in the profile or the absolute position of the profile along the Y-axis (*i.e.* the ΔG -axis) are absolutes.

Profiles that are shifted upward or downward (*i.e.* having on an average, higher or lower values at every position) but retain the relative shape and position of the profile along the X-axis can be foreseen as being equally effective as the model profile described here. Moreover, it is likely that siRNA that have strong or even stronger gene-specific silencing effects might have exaggerated ΔG values (either higher or lower) at key positions. Thus, for instance, it is possible that the 5'-most position of the sense strand (position 19) could have ΔG values of 7.4 kcal/mol or lower and still be a strong siRNA if, for instance, a G-C → G-T/U mismatch were substituted at position 19 and altered duplex stability. Similarly, position 12 and position 7 could have values above 8.3 kcal/mol and below 7.7 kcal/mole, respectively, without abating the silencing effectiveness of the molecule. Thus, for instance, at position 12, a stabilizing chemical modification (*e.g.* a chemical modification of the 2' position of the sugar backbone) could be added that increases the average internal stability at that position. Similarly, at position 7, mismatches similar to those described previously could be introduced that would lower the ΔG values at that position.

Lastly, it is important to note that while functional and non-functional siRNA were originally defined as those molecules having specific silencing properties, both broader or more limiting parameters can be used to define these molecules. As used herein, unless otherwise specified, "non-functional siRNA" are defined as those siRNA that induce less than 50% (<50%) target silencing, "semi-functional siRNA" induce 50-79% target silencing, "functional siRNA" are molecules that induce 80-95% gene silencing, and "highly-functional siRNA" are molecules that induce great than 95% gene silencing. These definitions are not intended to be rigid and can vary depending upon the design and needs of the application. For instance, it is possible that a researcher attempting to map a gene to a chromosome using a functional assay, may identify an siRNA that reduces gene activity by only 30%. While this level of gene silencing may be "non-functional" for *e.g.* therapeutic needs, it is sufficient for gene mapping purposes and is, under these uses and conditions, "functional." For these reasons, functional siRNA can be defined as those molecules having greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% silencing capabilities at 100nM transfection conditions. Similarly, depending upon the needs of the study and/or application, non-functional and semi-functional siRNA can be defined as

having different parameters. For instance, semi-functional siRNA can be defined as being those molecules that induce 20%, 30%, 40%, 50%, 60%, or 70% silencing at 100nM transfection conditions. Similarly, non-functional siRNA can be defined as being those molecules that silence gene expression by less than 70%, 60%, 50%, 40%, 30%, or less. Nonetheless, unless otherwise stated, the descriptions stated in the "Definitions" section of this text should be applied.

Functional attributes can be assigned to each of the key positions in the AISP of strong siRNA. The low 5' (sense strand) AISP values of strong siRNAs may be necessary for determining which end of the molecule enters the RISC complex. In contrast, the high and low AISP values observed in the central regions of the molecule may be critical for siRNA-target mRNA interactions and product release, respectively.

If the AISP values described above accurately define the thermodynamic parameters of strong siRNA, it would be expected that similar patterns would be observed in strong siRNA isolated from nature. Natural siRNAs exist in a harsh, RNase-rich environment and it can be hypothesized that only those siRNA that exhibit heightened affinity for RISC (*i.e.* siRNA that exhibit an average internal stability profile similar to those observed in strong siRNA) would survive in an intracellular environment. This hypothesis was tested using GFP-specific siRNA isolated from *N. benthamiana*. Llave *et al.* (2002) *Endogenous and Silencing-Associated Small RNAs in Plants*, The Plant Cell 14, 1605-1619, introduced long double-stranded GFP-encoding RNA into plants and subsequently re-isolated GFP-specific siRNA from the tissues. The AISP of fifty-nine of these GFP-siRNA were determined, averaged, and subsequently plotted alongside the AISP profile obtained from the cyclophilin B/DBI/ luciferase siRNA having >90% silencing properties (**Figure 6b**). Comparison of the two groups show that profiles are nearly identical. This finding validates the information provided by the internal stability profiles and demonstrates that: (1) the profile identified by analysis of the cyclophilin B/DBI/ luciferase siRNAs are not gene specific; and (2) AISP values can be used to search for strong siRNAs in a variety of species.

Both chemical modifications and base-pair mismatches can be incorporated into siRNA to alter the duplex's AISP and functionality. For instance, introduction of mismatches at positions 1 or 2 of the sense strand destabilized the 5'end of the sense strand and increases the functionality of the molecule (see Luc, **Figure 7**). Similarly, addition of 2'-O-methyl groups to positions 1 and 2 of the sense strand can also alter the AISP and (as a result) increase both the functionality of the molecule and eliminate off-target effects that results from sense strand homology with the unrelated targets **(Figures 8a, 8b).**

Rationale for Criteria in a Biological Context

The fate of siRNA in the RNAi pathway may be described in 5 major steps: (1) duplex recognition and pre-RISC complex formation; (2) ATP-dependent duplex unwinding/strand selection and RISC activation; (3) mRNA target identification; (4) mRNA cleavage, and (5) product release (Figure 1). Given the level of nucleic acid-protein interactions at each step, siRNA functionality is likely influenced by specific biophysical and molecular properties that promote efficient interactions within the context of the multi-component complexes. Indeed, the systematic analysis of the siRNA test set identified multiple factors that correlate well with functionality. When combined into a single algorithm, they proved to be very effective in selecting active siRNAs.

The factors described here may also be predictive of key functional associations important for each step in RNAi. For example, the potential formation of internal hairpin structures correlated negatively with siRNA functionality. Complementary strands with stable internal repeats are more likely to exist as stable hairpins thus decreasing the effective concentration of the functional duplex form. This suggests that the duplex is the preferred conformation for initial pre-RISC association. Indeed, although single complementary strands can induce gene silencing, the effective concentration required is at least two orders of magnitude higher than that of the duplex form.

siRNA-pre-RISC complex formation is followed by an ATP-dependent duplex unwinding step and "activation" of the RISC. The siRNA functionality was shown to correlate with overall low internal stability of the duplex and low internal stability of

the 3' sense end (or differential internal stability of the 3' sense compare to the 5' sense strand), which may reflect strand selection and entry into the RISC. Overall duplex stability and low internal stability at the 3' end of the sense strand were also correlated with siRNA functionality. Interestingly, siRNAs with very high and very low overall stability profiles correlate strongly with non-functional duplexes. One interpretation is that high internal stability prevents efficient unwinding while very low stability reduces siRNA target affinity and subsequent mRNA cleavage by the RISC.

Several criteria describe base preferences at specific positions of the sense strand and are even more intriguing when considering their potential mechanistic roles in target recognition and mRNA cleavage. Base preferences for A at position 19 of the sense strand but not C, are particularly interesting because they reflect the same base preferences observed for naturally occurring miRNA precursors. That is, among the reported miRNA precursor sequences 75% contain a U at position 1 which corresponds to an A in position 19 of the sense strand of siRNAs, while G was under-represented in this same position for miRNA precursors. These observations support the hypothesis that both miRNA precursors and siRNA duplexes are processed by very similar if not identical protein machinery. The functional interpretation of the predominance of a U/A base pair is that it promotes flexibility at the 5'antisense ends of both siRNA duplexes and miRNA precursors and facilitates efficient unwinding and selective strand entrance into an activated RISC.

Among the criteria associated with base preferences that are likely to influence mRNA cleavage or possibly product release, the preference for U at position 10 of the sense strand exhibited the greatest impact, enhancing the probability of selecting an F80 sequence by 13.3%. Activated RISC preferentially cleaves target mRNA between nucleotides 10 and 11 relative to the 5' end of the complementary targeting strand. Therefore, it may be that U, the preferred base for most endoribonucleases, at this position supports more efficient cleavage. Alternatively, a U/A bp between the targeting siRNA strand and its cognate target mRNA may create an optimal conformation for the RISC-associated "slicing" activity.

Pooling

According to another embodiment, the present invention provides a pool of at least two siRNAs, preferably in the form of a kit or therapeutic reagent, wherein one strand of each of the siRNAs, the sense strand comprises a sequence that is substantially similar to a sequence within a target mRNA. The opposite strand, the antisense strand, will preferably comprise a sequence that is substantially complementary to that of the target mRNA. More preferably, one strand of each siRNA will comprise a sequence that is identical to a sequence that is contained in the target mRNA. Most preferably, each siRNA will be 19 base pairs in length, and one strand of each of the siRNAs will be 100% complementary to a portion of the target mRNA.

By increasing the number of siRNAs directed to a particular target using a pool or kit, one is able both to increase the likelihood that at least one siRNA with satisfactory functionality will be included, as well as to benefit from additive or synergistic effects. Further, when two or more siRNAs directed against a single gene do not have satisfactory levels of functionality alone, if combined, they may satisfactorily promote degradation of the target messenger RNA and successfully inhibit translation. By including multiple siRNAs in the system, not only is the probability of silencing increased, but the economics of operation are also improved when compared to adding different siRNAs sequentially. This effect is contrary to the conventional wisdom that the concurrent use of multiple siRNA will negatively impact gene silencing (*e.g.* Holen, T. *et al.*,(2003) "Similar behavior of single strand and double strand siRNAs suggests they act through a common RNAi pathway." NAR 31: 2401-21407).

In fact, when two siRNAs were pooled together, 54% of the pools of two siRNAs induced more than 95% gene silencing. Thus, a 2.5-fold increase in the percentage of functionality was achieved by randomly combining two siRNAs. Further, over 84% of pools containing two siRNAs induced more than 80% gene silencing.

More preferably, the kit is comprised of at least three siRNAs, wherein one strand of each siRNA comprises a sequence that is substantially similar to a sequence

of the target mRNA and the other strand comprises a sequence that is substantially complementary to the region of the target mRNA. As with the kit that comprises at least two siRNAs, more preferably one strand will comprise a sequence that is identical to a sequence that is contained in the mRNA and another strand that is 100% complementary to a sequence that is contained in the mRNA. During experiments, when three siRNAs were combined together, 60% of the pools induced more than 95% gene silencing and 92% of the pools induced more than 80% gene silencing.

Further, even more preferably, the kit is comprised of at least four siRNAs, wherein one strand of each siRNA comprises a sequence that is substantially similar to a region of the sequence of the target mRNA, and the other strand comprises a sequence that is substantially complementary to the region of the target mRNA. As with the kit or pool that comprises at least two siRNAs, more preferably one strand of each of the siRNA duplexes will comprise a sequence that is identical to a sequence that is contained in the mRNA, and another strand that is 100% complementary to a sequence that is contained in the mRNA.

Additionally, kits and pools with at least five, at least six, and at least seven siRNAs may also be useful with the present invention. For example, pools of five siRNA induced 95% gene silencing with 77% probability and 80% silencing with 98.8% probability. Thus, pooling of siRNAs together can result in the creation of a target-specific silencing reagent with almost a 99% probability of being functional. The fact that such high levels of success are achievable using such pools of siRNA, enables one to dispense with costly and time-consuming target-specific validation procedures.

For this embodiment, as well as the other aforementioned embodiments, each of the siRNAs within a pool will preferably comprise between 18 and 30 base pairs, more preferably between 18 and 25 base pairs, and most preferably 19 base pairs. Within each siRNA, preferably at least 18 contiguous bases of the antisense strand will be 100% complementary to the target mRNA. More preferably, at least 19 contiguous bases of the antisense strand will be 100% complementary to the target mRNA. Additionally, there may be overhangs on either the sense strand or the antisense strand, and these overhangs may be at either the 5' end or the 3' end of

either of the strands, for example there may be one or more overhangs of 1-6 bases. When overhangs are present, they are not included in the calculation of the number of base pairs. The two nucleotide 3' overhangs mimic natural siRNAs and are commonly used but are not essential. Preferably, the overhangs should consist of two nucleotides, most often dTdT or UU at the 3' end of the sense and antisense strand that are not complementary to the target sequence. The siRNAs may be produced by any method that is now known or that comes to be known for synthesizing double stranded RNA that one skilled in the art would appreciate would be useful in the present invention. Preferably, the siRNAs will be produced by Dharmacon's proprietary ACE® technology. However, other methods for synthesizing siRNAs are well known to persons skilled in the art and include, but are not limited to, any chemical synthesis of RNA oligonucleotides, ligation of shorter oligonucleotides, *in vitro* transcription of RNA oligonucleotides, the use of vectors for expression within cells, recombinant Dicer products and PCR products.

The siRNA duplexes within the aforementioned pools of siRNAs may correspond to overlapping sequences within a particular mRNA, or non-overlapping sequences of the mRNA. However, preferably they correspond to non-overlapping sequences. Further, each siRNA may be selected randomly, or one or more of the siRNA may be selected according to the criteria discussed above for maximizing the effectiveness of siRNA.

Included in the definition of siRNAs are siRNAs that contain substituted and/or labeled nucleotides that may, for example, be labeled by radioactivity, fluorescence or mass. The most common substitutions are at the 2' position of the ribose sugar, where moieties such as H (hydrogen) F, NH_3, OCH_3 and other O- alkyl, alkenyl, alkynyl, and orthoesters, may be substituted, or in the phosphorous backbone, where sulfur, amines or hydrocarbons may be substituted for the bridging of non-bridging atoms in the phosphodiester bond. Examples of modified siRNAs are explained more fully in commonly assigned U.S. Patent Application Ser. No. 10/613,077, filed July 1, 2003, which is incorporated by reference herein.

Additionally, as noted above, the cell type into which the siRNA is introduced may affect the ability of the siRNA to enter the cell; however, it does not appear to

affect the ability of the siRNA to function once it enters the cell. Methods for introducing double-stranded RNA into various cell types are well known to persons skilled in the art.

As persons skilled in the art are aware, in certain species, the presence of proteins such as RdRP, the RNA-dependent RNA polymerase, may catalytically enhance the activity of the siRNA. For example, RdRP propagates the RNAi effect in *C. elegans* and other non-mammalian organisms. In fact, in organisms that contain these proteins, the siRNA may be inherited. Two other proteins that are well studied and known to be a part of the machinery are members of the Argonaute family and Dicer, as well as their homologues. There is also initial evidence that the RISC complex might be associated with the ribosome so the more efficiently translated mRNAs will be more susceptible to silencing than others.

Another very important factor in the efficacy of siRNA is mRNA localization. In general, only cytoplasmic mRNAs are considered to be accessible to RNAi to any appreciable degree. However, appropriately designed siRNAs, for example, siRNAs modified with internucleotide linkages, may be able to cause silencing by acting in the nucleus. Examples of these types of modifications are described in commonly assigned U.S. Patent Application Serial Nos. 10/431,027 and 10/613,077, each of which is incorporated by reference herein.

As described above, even when one selects at least two siRNAs at random, the effectiveness of the two may be greater than one would predict based on the effectiveness of two individual siRNAs. This additive or synergistic effect is particularly noticeable as one increases to at least three siRNAs, and even more noticeable as one moves to at least four siRNAs. Surprisingly, the pooling of the non-functional and semi-functional siRNAs, particularly more than five siRNAs, can lead to a silencing mixture that is as effective if not more effective than any one particular functional siRNA.

Within the kit of the present invention, preferably each siRNA will be present in a concentration of between 0.001 and 200 μM, more preferably between 0.01 and 200 nM, and most preferably between 0.1 and 10 nM.

In addition to preferably comprising at least four or five siRNAs, the kit of the present invention will also preferably comprise a buffer to keep the siRNA duplex stable. Persons skilled in the art are aware of buffers suitable for keeping siRNA stable. For example, the buffer may be comprised of 100 mM KCl, 30 mM HEPES-pH 7.5, and 1 mM $MgCl_2$. Alternatively, kits might contain complementary strands that contain any one of a number of chemical modifications (*e.g.* a 2'-O-ACE) that protect the agents from degradation by nucleases. In this instance, the user may (or may not) remove the modifying protective group (*e.g.* deprotect) before annealing the two complementary strands together.

By way of example, the kit may be organized such that pools of siRNA duplexes are provided on an array or microarray of wells or drops for a particular gene set or for unrelated genes. The array may, for example, be in 96 wells, 384 wells or 1284 wells arrayed in a plastic plate or on a glass slide using techniques now known or that come to be known to persons skilled in the art. Within an array, preferably there will be controls such as functional anti-lamin A/C, cyclophilin and two siRNA duplexes that are not specific to the gene of interest.

In order to ensure stability of the siRNA pools prior to usage, they may be retained in lyophilized form at minus twenty degrees (−20°C) until they are ready for use. Prior to usage, they should be resuspended; however, even once resuspended, for example, in the aforementioned buffer, they should be kept at minus twenty degrees, (−20°C) until used. The aforementioned buffer, prior to use, may be stored at approximately 4°C or room temperature. Effective temperatures at which to conduct transfections are well known to persons skilled in the art and include for example, room temperature.

The kit may be applied either *in vivo* or *in vitro*. Preferably, the siRNA of the pools or kits is applied to a cell through transfection, employing standard transfection

protocols. These methods are well known to persons skilled in the art and include the use of lipid-based carriers, electroporation, cationic carriers, and microinjection. Further, one could apply the present invention by synthesizing equivalent DNA sequences (either as two separate, complementary strands, or as hairpin molecules) instead of siRNA sequences and introducing them into cells through vectors. Once in the cells, the cloned DNA could be transcribed, thereby forcing the cells to generate the siRNA. Examples of vectors suitable for use with the present application include but are not limited to the standard transient expression vectors, adenoviruses, retroviruses, lentivirus-based vectors, as well as other traditional expression vectors. Any vector that has an adequate siRNA expression and procession module may be used. Furthermore, certain chemical modifications to siRNAs, including but not limited to conjugations to other molecules, may be used to facilitate delivery. For certain applications it may be preferable to deliver molecules without transfection by simply formulating in a physiological acceptable solution.

This embodiment may be used in connection with any of the aforementioned embodiments. Accordingly, the sequences within any pool may be selected by rational design.

Multigene Silencing

In addition to developing kits that contain multiple siRNA directed against a single gene, another embodiment includes the use of multiple siRNA targeting multiple genes. Multiple genes may be targeted through the use of high- or hyper-functional siRNA. High- or hyper- functional siRNA that exhibit increased potency, require lower concentrations to induce desired phenotypic (and thus therapeutic) effects. This circumvents RISC saturation. It therefore reasons that if lower concentrations of a single siRNA are needed for knockout or knockdown expression of one gene, then the remaining (uncomplexed) RISC will be free and available to interact with siRNA directed against two, three, four, or more, genes. Thus in this embodiment, the authors describe the use of highly functional or hyper-functional siRNA to knock out three separate genes. More preferably, such reagents could be combined to knockout four distinct genes. Even more preferably, highly functional or hyperfunctional siRNA could be used to knock out five distinct genes. Most

preferably, siRNA of this type could be used to knockout or knockdown the expression of six or more genes.

Hyperfunctional siRNA

The term hyperfunctional siRNA (hf-siRNA) describes a subset of the siRNA population that induces RNAi in cells at low- or sub-nanomolar concentrations for extended periods of time. These traits, heightened potency and extended longevity of the RNAi phenotype, are highly attractive from a therapeutic standpoint. Agents having higher potency require lesser amounts of the molecule to achieve the desired physiological response, thus reducing the probability of side effects due to "off-target" interference. In addition to the potential therapeutic benefits associated with hyperfunctional siRNA, hf-siRNA are also desirable from an economic position. Hyperfunctional siRNA may cost less on a per-treatment basis, thus reducing the overall expenditures to both the manufacturer and the consumer.

Identification of hyperfunctional siRNA involves multiple steps that are designed to examine an individual siRNA agent's concentration- and/or longevity-profiles. In one non-limiting example, a population of siRNA directed against a single gene are first analyzed using the previously described algorithm (Formula VIII). Individual siRNA are then introduced into a test cell line and assessed for the ability to degrade the target mRNA. It is important to note that when performing this step it is not necessary to test all of the siRNA. Instead, it is sufficient to test only those siRNA having the highest SMARTscores™ (*i.e.* SMARTscore™ > -10). Subsequently, the gene silencing data is plotted against the SMARTscores™ (see **Figure 9**). SiRNA that (1) induce a high degree of gene silencing (*i.e.* they induce greater than 80% gene knockdown) and (2) have superior SMARTscores™ (*i.e.* a SMARTscore™ of > -10, suggesting a desirable average internal stability profile) are selected for further investigations designed to better understand the molecule's potency and longevity. In one, non-limiting study dedicated to understanding a molecule's potency, an siRNA is introduced into one (or more) cell types in increasingly diminishing concentrations (*e.g.* 3.0 → 0.3 nM). Subsequently, the level of gene silencing induced by each concentration is examined and siRNA that exhibit hyperfunctional potency (*i.e.* those that induce 80% silencing or greater at *e.g.* picomolar concentrations) are identified. In a second study, the longevity profiles of

siRNA having high (>-10) SMARTscores™ and greater than 80% silencing are examined. In one non-limiting example of how this is achieved, siRNA are introduced into a test cell line and the levels of RNAi are measured over an extended period of time (*e.g.* 24-168 hrs). SiRNAs that exhibit strong RNA interference patterns (*i.e.* >80 % interference) for periods of time greater than, *e.g.,* 120 hours are thus identified. Studies similar to those described above can be performed on any and all of the $>10^6$ siRNA included in this document to further define the most functional molecule for any given gene. Molecules possessing one or both properties (extended longevity and heightened potency) are labeled "hyperfunctional siRNA," and earmarked as candidates for future therapeutic studies.

While the example(s) given above describe one means by which hyperfunctional siRNA can be isolated, neither the assays themselves nor the selection parameters used are rigid and can vary with each family of siRNA. Families of siRNA include siRNAs directed against a single gene, or directed against a related family of genes.

The highest quality siRNA achievable for any given gene may vary considerably. Thus, for example, in the case of one gene (gene X), rigorous studies such as those described above may enable the identification of an siRNA that, at picomolar concentrations, induces 99+% silencing for a period of 10 days. Yet identical studies of a second gene (gene Y) may yield an siRNA that at high nanomolar concentrations (*e.g.* 100nM) induces only 75% silencing for a period of 2 days. Both molecules represent the very optimum siRNA for their respective gene targets and therefore are designated "hyperfunctional." Yet due to a variety of factors including but not limited to target concentration, siRNA stability, cell type, off-target interference, and others, equivalent levels of potency and longevity are not achievable. Thus, for these reasons, the parameters described in the before mentioned assays, can vary. While the initial screen selected siRNA that had SMARTscores™ above –10 and a gene silencing capability of greater than 80%, selections that have stronger (or weaker) parameters can be implemented. Similarly, in the subsequent studies designed to identify molecules with high potency and longevity, the desired cutoff criteria (*i.e.* the lowest concentration that induces a desirable level of interference, or the longest period of time that interference can be observed) can vary.

The experimentation subsequent to application of the rational criteria of this application is significantly reduced where one is trying to obtain a suitable hyperfunctional siRNA for, for example, therapeutic use. When, for example, the additional experimentation of the type described herein is applied by one skilled in the art with this disclosure in hand, a hyperfunctional siRNA is readily identified.

The siRNA may be introduced into a cell by any method that is now known or that comes to be known and that from reading this disclosure, persons skilled in the art would determine would be useful in connection with the present invention in enabling siRNA to cross the cellular membrane. These methods include, but are not limited to, any manner of transfection, such as for example transfection employing DEAE-Dextran, calcium phosphate, cationic lipids/liposomes, micelles, manipulation of pressure, microinjection, electroporation, immunoporation, use of vectors such as viruses, plasmids, cosmids, bacteriophages, cell fusions, and coupling of the polynucleotides to specific conjugates or ligands such as antibodies, antigens, or receptors, passive introduction, adding moieties to the siRNA that facilitate its uptake, and the like.

Having described the invention with a degree of particularity, examples will now be provided. These examples are not intended to and should not be construed to limit the scope of the claims in any way.

Examples

General Techniques and Nomenclatures

siRNA nomenclature. All siRNA duplexes are referred to by sense strand. The first nucleotide of the 5'-end of the sense strand is position 1, which corresponds to position 19 of the antisense strand for a 19-mer. In most cases, to compare results from different experiments, silencing was determined by measuring specific transcript mRNA levels or enzymatic activity associated with specific transcript levels, 24 hours post-transfection, with siRNA concentrations held constant at 100 nM. For all experiments, unelss otherwise specified transfection efficiency was ensured to be over 95%, and no detectable cellular toxicity was observed. The following system of nomenclature was used to compare and report siRNA-silencing functionality: "F"

followed by the degree of minimal knockdown. For example, F50 signifies at least 50% knockdown, F80 means at least 80%, and so forth. For this study, all sub-F50 siRNAs were considered non-functional.

Cell culture and transfection. 96-well plates are coated with 50 µl of 50 mg/ml poly-L-lysine (Sigma) for 1 hr, and then washed 3X with distilled water before being dried for 20 min. HEK293 cells or HEK293Lucs or any other cell type of interest are released from their solid support by trypsinization, diluted to 3.5 X 10^5 cells/ml, followed by the addition of 100 µL of cells/well. Plates are then incubated overnight at 37° C, 5% CO_2. Transfection procedures can vary widely depending on the cell type and transfection reagents. In one non-limiting example, a transfection mixture consisting of 2 mL Opti-MEM I (Gibco-BRL), 80 µl Lipofectamine 2000 (Invitrogen), 15 µL SUPERNasin at 20 U/µl (Ambion), and 1.5 µl of reporter gene plasmid at 1 µg/ µl is prepared in 5-ml polystyrene round bottom tubes. 100 µl of transfection reagent is then combined with 100 µl of siRNAs in polystyrene deep-well titer plates (Beckman) and incubated for 20 to 30 min at room temp. 550 µl of Opti-MEM is then added to each well to bring the final siRNA concentration to 100 nM. Plates are then sealed with parafilm and mixed. Media is removed from HEK293 cells and replaced with 95 µl of transfection mixture. Cells are incubated overnight at 37° C, 5% CO_2.

Quantification of gene knockdown. A variety of quantification procedures can be used to measure the level of silencing induced by siRNA or siRNA pools. In one non-limiting example: to measure mRNA levels 24 hrs post-transfection, QuantiGene branched-DNA (bDNA) kits (Bayer) (Wang, *et al, Regulation of insulin preRNA splicing by glucose.* Proc Natl Acad Sci 1997, 94:4360.) are used according to manufacturer instructions. To measure luciferase activity, media is removed from HEK293 cells 24 hrs post-transfection, and 50 µl of Steady-GLO reagent (Promega) is added. After 5 min, plates are analyzed on a plate reader.

Example I. Sequences Used to Develop the Algorithm.

Anti-Firefly and anti-Cyclophilin siRNAs panels **(Figure 5a, b)** sorted according to using Formula VIII predicted values. All siRNAs scoring more than 0

(formula VIII) and more then 20 (formula IX) are fully functional. All ninety sequences for each gene (and DBI) appear below in **Table III**.

TABLE III

Cyclo	1	SEQ. ID 0032	GUUCCAAAAACAGUGGAUA
Cyclo	2	SEQ. ID 0033	UCCAAAAACAGUGGAUAAU
Cyclo	3	SEQ. ID 0034	CAAAAACAGUGGAUAAUUU
Cyclo	4	SEQ. ID 0035	AAAACAGUGGAUAAUUUUG
Cyclo	5	SEQ. ID 0036	AACAGUGGAUAAUUUUGUG
Cyclo	6	SEQ. ID 0037	CAGUGGAUAAUUUUGUGGC
Cyclo	7	SEQ. ID 0038	GUGGAUAAUUUUGUGGCCU
Cyclo	8	SEQ. ID 0039	GGAUAAUUUUGUGGCCUUA
Cyclo	9	SEQ. ID 0040	AUAAUUUUGUGGCCUUAGC
Cyclo	10	SEQ. ID 0041	AAUUUUGUGGCCUUAGCUA
Cyclo	11	SEQ. ID 0042	UUUUGUGGCCUUAGCUACA
Cyclo	12	SEQ. ID 0043	UUGUGGCCUUAGCUACAGG
Cyclo	13	SEQ. ID 0044	GUGGCCUUAGCUACAGGAG
Cyclo	14	SEQ. ID 0045	GGCCUUAGCUACAGGAGAG
Cyclo	15	SEQ. ID 0046	CCUUAGCUACAGGAGAGAA
Cyclo	16	SEQ. ID 0047	UUAGCUACAGGAGAGAAAG
Cyclo	17	SEQ. ID 0048	AGCUACAGGAGAGAAAGGA
Cyclo	18	SEQ. ID 0049	CUACAGGAGAGAAAGGAUU
Cyclo	19	SEQ. ID 0050	ACAGGAGAGAAAGGAUUUG
Cyclo	20	SEQ. ID 0051	AGGAGAGAAAGGAUUUGGC
Cyclo	21	SEQ. ID 0052	GAGAGAAAGGAUUUGGCUA
Cyclo	22	SEQ. ID 0053	GAGAAAGGAUUUGGCUACA
Cyclo	23	SEQ. ID 0054	GAAAGGAUUUGGCUACAAA
Cyclo	24	SEQ. ID 0055	AAGGAUUUGGCUACAAAAA
Cyclo	25	SEQ. ID 0056	GGAUUUGGCUACAAAAACA
Cyclo	26	SEQ. ID 0057	AUUUGGCUACAAAAACAGC
Cyclo	27	SEQ. ID 0058	UUGGCUACAAAAACAGCAA
Cyclo	28	SEQ. ID 0059	GGCUACAAAAACAGCAAAU
Cyclo	29	SEQ. ID 0060	CUACAAAAACAGCAAAUUC
Cyclo	30	SEQ. ID 0061	ACAAAAACAGCAAAUUCCA
Cyclo	31	SEQ. ID 0062	AAAAACAGCAAAUUCCAUC
Cyclo	32	SEQ. ID 0063	AAACAGCAAAUUCCAUCGU
Cyclo	33	SEQ. ID 0064	ACAGCAAAUUCCAUCGUGU
Cyclo	34	SEQ. ID 0065	AGCAAAUUCCAUCGUGUAA

Cyclo	35	SEQ. ID 0066	CAAAUUCCAUCGUGUAAUC
Cyclo	36	SEQ. ID 0067	AAUUCCAUCGUGUAAUCAA
Cyclo	37	SEQ. ID 0068	UUCCAUCGUGUAAUCAAGG
Cyclo	38	SEQ. ID 0069	CCAUCGUGUAAUCAAGGAC
Cyclo	39	SEQ. ID 0070	AUCGUGUAAUCAAGGACUU
Cyclo	40	SEQ. ID 0071	CGUGUAAUCAAGGACUUCA
Cyclo	41	SEQ. ID 0072	UGUAAUCAAGGACUUCAUG
Cyclo	42	SEQ. ID 0073	UAAUCAAGGACUUCAUGAU
Cyclo	43	SEQ. ID 0074	AUCAAGGACUUCAUGAUCC
Cyclo	44	SEQ. ID 0075	CAAGGACUUCAUGAUCCAG
Cyclo	45	SEQ. ID 0076	AGGACUUCAUGAUCCAGGG
Cyclo	46	SEQ. ID 0077	GACUUCAUGAUCCAGGGCG
Cyclo	47	SEQ. ID 0078	CUUCAUGAUCCAGGGCGGA
Cyclo	48	SEQ. ID 0079	UCAUGAUCCAGGGCGGAGA
Cyclo	49	SEQ. ID 0080	AUGAUCCAGGGCGGAGACU
Cyclo	50	SEQ. ID 0081	GAUCCAGGGCGGAGACUUC
Cyclo	51	SEQ. ID 0082	UCCAGGGCGGAGACUUCAC
Cyclo	52	SEQ. ID 0083	CAGGGCGGAGACUUCACCA
Cyclo	53	SEQ. ID 0084	GGGCGGAGACUUCACCAGG
Cyclo	54	SEQ. ID 0085	GCGGAGACUUCACCAGGGG
Cyclo	55	SEQ. ID 0086	GGAGACUUCACCAGGGGAG
Cyclo	56	SEQ. ID 0087	AGACUUCACCAGGGGAGAU
Cyclo	57	SEQ. ID 0088	ACUUCACCAGGGGAGAUGG
Cyclo	58	SEQ. ID 0089	UUCACCAGGGGAGAUGGCA
Cyclo	59	SEQ. ID 0090	CACCAGGGGAGAUGGCACA
Cyclo	60	SEQ. ID 0091	CCAGGGGAGAUGGCACAGG
Cyclo	61	SEQ. ID 0092	AGGGGAGAUGGCACAGGAG
Cyclo	62	SEQ. ID 0093	GGGAGAUGGCACAGGAGGA
Cyclo	63	SEQ. ID 0094	GAGAUGGCACAGGAGGAAA
Cyclo	64	SEQ. ID 0095	GAUGGCACAGGAGGAAAGA
Cyclo	65	SEQ. ID 0094	UGGCACAGGAGGAAAGAGC
Cyclo	66	SEQ. ID 0096	GCACAGGAGGAAAGAGCAU
Cyclo	67	SEQ. ID 0097	ACAGGAGGAAAGAGCAUCU
Cyclo	68	SEQ. ID 0098	AGGAGGAAAGAGCAUCUAC
Cyclo	69	SEQ. ID 0099	GAGGAAAGAGCAUCUACGG
Cyclo	70	SEQ. ID 0100	GGAAAGAGCAUCUACGGUG
Cyclo	71	SEQ. ID 0101	AAAGAGCAUCUACGGUGAG
Cyclo	72	SEQ. ID 0102	AGAGCAUCUACGGUGAGCG
Cyclo	73	SEQ. ID 0103	AGCAUCUACGGUGAGCGCU

Cyclo	74	SEQ. ID 0104	CAUCUACGGUGAGCGCUUC
Cyclo	75	SEQ. ID 0105	UCUACGGUGAGCGCUUCCC
Cyclo	76	SEQ. ID 0106	UACGGUGAGCGCUUCCCCG
Cyclo	77	SEQ. ID 0107	CGGUGAGCGCUUCCCCGAU
Cyclo	78	SEQ. ID 0108	GUGAGCGCUUCCCCGAUGA
Cyclo	79	SEQ. ID 0109	GAGCGCUUCCCCGAUGAGA
Cyclo	80	SEQ. ID 0110	GCGCUUCCCCGAUGAGAAC
Cyclo	81	SEQ. ID 0111	GCUUCCCCGAUGAGAACUU
Cyclo	82	SEQ. ID 0112	UUCCCCGAUGAGAACUUCA
Cyclo	83	SEQ. ID 0113	CCCCGAUGAGAACUUCAAA
Cyclo	84	SEQ. ID 0114	CCGAUGAGAACUUCAAACU
Cyclo	85	SEQ. ID 0115	GAUGAGAACUUCAAACUGA
Cyclo	86	SEQ. ID 0116	UGAGAACUUCAAACUGAAG
Cyclo	87	SEQ. ID 0117	AGAACUUCAAACUGAAGCA
Cyclo	88	SEQ. ID 0118	AACUUCAAACUGAAGCACU
Cyclo	89	SEQ. ID 0119	CUUCAAACUGAAGCACUAC
Cyclo	90	SEQ. ID 0120	UCAAACUGAAGCACUACGG
DB	1	SEQ. ID 0121	ACGGGCAAGGCCAAGUGGG
DB	2	SEQ. ID 0122	CGGGCAAGGCCAAGUGGGA
DB	3	SEQ. ID 0123	GGGCAAGGCCAAGUGGGAU
DB	4	SEQ. ID 0124	GGCAAGGCCAAGUGGGAUG
DB	5	SEQ. ID 0125	GCAAGGCCAAGUGGGAUGC
DB	6	SEQ. ID 0126	CAAGGCCAAGUGGGAUGCC
DB	7	SEQ. ID 0127	AAGGCCAAGUGGGAUGCCU
DB	8	SEQ. ID 0128	AGGCCAAGUGGGAUGCCUG
DB	9	SEQ. ID 0129	GGCCAAGUGGGAUGCCUGG
DB	10	SEQ. ID 0130	GCCAAGUGGGAUGCCUGGA
DB	11	SEQ. ID 0131	CCAAGUGGGAUGCCUGGAA
DB	12	SEQ. ID 0132	CAAGUGGGAUGCCUGGAAU
DB	13	SEQ. ID 0133	AAGUGGGAUGCCUGGAAUG
DB	14	SEQ. ID 0134	AGUGGGAUGCCUGGAAUGA
DB	15	SEQ. ID 0135	GUGGGAUGCCUGGAAUGAG
DB	16	SEQ. ID 0136	UGGGAUGCCUGGAAUGAGC
DB	17	SEQ. ID 0137	GGGAUGCCUGGAAUGAGCU
DB	18	SEQ. ID 0138	GGAUGCCUGGAAUGAGCUG
DB	19	SEQ. ID 0139	GAUGCCUGGAAUGAGCUGA
DB	20	SEQ. ID 0140	AUGCCUGGAAUGAGCUGAA
DB	21	SEQ. ID 0141	UGCCUGGAAUGAGCUGAAA
DB	22	SEQ. ID 0142	GCCUGGAAUGAGCUGAAAG
DB	23	SEQ. ID 0143	CCUGGAAUGAGCUGAAAGG

DB	24	SEQ. ID 0144	CUGGAAUGAGCUGAAAGGG
DB	25	SEQ. ID 0145	UGGAAUGAGCUGAAAGGGA
DB	26	SEQ. ID 0146	GGAAUGAGCUGAAAGGGAC
DB	27	SEQ. ID 0147	GAAUGAGCUGAAAGGGACU
DB	28	SEQ. ID 0148	AAUGAGCUGAAAGGGACUU
DB	29	SEQ. ID 0149	AUGAGCUGAAAGGGACUUC
DB	30	SEQ. ID 0150	UGAGCUGAAAGGGACUUCC
DB	31	SEQ. ID 0151	GAGCUGAAAGGGACUUCCA
DB	32	SEQ. ID 0152	AGCUGAAAGGGACUUCCAA
DB	33	SEQ. ID 0153	GCUGAAAGGGACUUCCAAG
DB	34	SEQ. ID 0154	CUGAAAGGGACUUCCAAGG
DB	35	SEQ. ID 0155	UGAAAGGGACUUCCAAGGA
DB	36	SEQ. ID 0156	GAAAGGGACUUCCAAGGAA
DB	37	SEQ. ID 0157	AAAGGGACUUCCAAGGAAG
DB	38	SEQ. ID 0158	AAGGGACUUCCAAGGAAGA
DB	39	SEQ. ID 0159	AGGGACUUCCAAGGAAGAU
DB	40	SEQ. ID 0160	GGGACUUCCAAGGAAGAUG
DB	41	SEQ. ID 0161	GGACUUCCAAGGAAGAUGC
DB	42	SEQ. ID 0162	GACUUCCAAGGAAGAUGCC
DB	43	SEQ. ID 0163	ACUUCCAAGGAAGAUGCCA
DB	44	SEQ. ID 0164	CUUCCAAGGAAGAUGCCAU
DB	45	SEQ. ID 0165	UUCCAAGGAAGAUGCCAUG
DB	46	SEQ. ID 0166	UCCAAGGAAGAUGCCAUGA
DB	47	SEQ. ID 0167	CCAAGGAAGAUGCCAUGAA
DB	48	SEQ. ID 0168	CAAGGAAGAUGCCAUGAAA
DB	49	SEQ. ID 0169	AAGGAAGAUGCCAUGAAAG
DB	50	SEQ. ID 0170	AGGAAGAUGCCAUGAAAGC
DB	51	SEQ. ID 0171	GGAAGAUGCCAUGAAAGCU
DB	52	SEQ. ID 0172	GAAGAUGCCAUGAAAGCUU
DB	53	SEQ. ID 0173	AAGAUGCCAUGAAAGCUUA
DB	54	SEQ. ID 0174	AGAUGCCAUGAAAGCUUAC
DB	55	SEQ. ID 0175	GAUGCCAUGAAAGCUUACA
DB	56	SEQ. ID 0176	AUGCCAUGAAAGCUUACAU
DB	57	SEQ. ID 0177	UGCCAUGAAAGCUUACAUC
DB	58	SEQ. ID 0178	GCCAUGAAAGCUUACAUCA
DB	59	SEQ. ID 0179	CCAUGAAAGCUUACAUCAA
DB	60	SEQ. ID 0180	CAUGAAAGCUUACAUCAAC
DB	61	SEQ. ID 0181	AUGAAAGCUUACAUCAACA
DB	62	SEQ. ID 0182	UGAAAGCUUACAUCAACAA
DB	63	SEQ. ID 0183	GAAAGCUUACAUCAACAAA

DB	64	SEQ. ID 0184	AAAGCUUACAUCAACAAAG
DB	65	SEQ. ID 0185	AAGCUUACAUCAACAAAGU
DB	66	SEQ. ID 0186	AGCUUACAUCAACAAAGUA
DB	67	SEQ. ID 0187	GCUUACAUCAACAAAGUAG
DB	68	SEQ. ID 0188	CUUACAUCAACAAAGUAGA
DB	69	SEQ. ID 0189	UUACAUCAACAAAGUAGAA
DB	70	SEQ. ID 0190	UACAUCAACAAAGUAGAAG
DB	71	SEQ. ID 0191	ACAUCAACAAAGUAGAAGA
DB	72	SEQ. ID 0192	CAUCAACAAAGUAGAAGAG
DB	73	SEQ. ID 0193	AUCAACAAAGUAGAAGAGC
DB	74	SEQ. ID 0194	UCAACAAAGUAGAAGAGCU
DB	75	SEQ. ID 0195	CAACAAAGUAGAAGAGCUA
DB	76	SEQ. ID 0196	AACAAAGUAGAAGAGCUAA
DB	77	SEQ. ID 0197	ACAAAGUAGAAGAGCUAAA
DB	78	SEQ. ID 0198	CAAAGUAGAAGAGCUAAAG
DB	79	SEQ. ID 0199	AAAGUAGAAGAGCUAAAGA
DB	80	SEQ. ID 0200	AAGUAGAAGAGCUAAAGAA
DB	81	SEQ. ID 0201	AGUAGAAGAGCUAAAGAAA
DB	82	SEQ. ID 0202	GUAGAAGAGCUAAAGAAAA
DB	83	SEQ. ID 0203	UAGAAGAGCUAAAGAAAAA
DB	84	SEQ. ID 0204	AGAAGAGCUAAAGAAAAAA
DB	85	SEQ. ID 0205	GAAGAGCUAAAGAAAAAAU
DB	86	SEQ. ID 0206	AAGAGCUAAAGAAAAAAUA
DB	87	SEQ. ID 0207	AGAGCUAAAGAAAAAAUAC
DB	88	SEQ. ID 0208	GAGCUAAAGAAAAAAUACG
DB	89	SEQ. ID 0209	AGCUAAAGAAAAAAUACGG
DB	90	SEQ. ID 0210	GCUAAAGAAAAAAUACGGG
Luc	1	SEQ. ID 0211	AUCCUCAUAAAGGCCAAGA
Luc	2	SEQ. ID 0212	AGAUCCUCAUAAAGGCCAA
Luc	3	SEQ. ID 0213	AGAGAUCCUCAUAAAGGCC
Luc	4	SEQ. ID 0214	AGAGAGAUCCUCAUAAAGG
Luc	5	SEQ. ID 0215	UCAGAGAGAUCCUCAUAAA
Luc	6	SEQ. ID 0216	AAUCAGAGAGAUCCUCAUA
Luc	7	SEQ. ID 0217	AAAAUCAGAGAGAUCCUCA
Luc	8	SEQ. ID 0218	GAAAAAUCAGAGAGAUCCU
Luc	9	SEQ. ID 0219	AAGAAAAAUCAGAGAGAUC
Luc	10	SEQ. ID 0220	GCAAGAAAAAUCAGAGAGA
Luc	11	SEQ. ID 0221	ACGCAAGAAAAAUCAGAGA
Luc	12	SEQ. ID 0222	CGACGCAAGAAAAAUCAGA
Luc	13	SEQ. ID 0223	CUCGACGCAAGAAAAAUCA

Luc	14	SEQ. ID 0224	AACUCGACGCAAGAAAAAU
Luc	15	SEQ. ID 0225	AAAACUCGACGCAAGAAAA
Luc	16	SEQ. ID 0226	GGAAAACUCGACGCAAGAA
Luc	17	SEQ. ID 0227	CCGGAAAACUCGACGCAAG
Luc	18	SEQ. ID 0228	UACCGGAAAACUCGACGCA
Luc	19	SEQ. ID 0229	CUUACCGGAAAACUCGACG
Luc	20	SEQ. ID 0230	GUCUUACCGGAAAACUCGA
Luc	21	SEQ. ID 0231	AGGUCUUACCGGAAAACUC
Luc	22	SEQ. ID 0232	AAAGGUCUUACCGGAAAAC
Luc	23	SEQ. ID 0233	CGAAAGGUCUUACCGGAAA
Luc	24	SEQ. ID 0234	ACCGAAAGGUCUUACCGGA
Luc	25	SEQ. ID 0235	GUACCGAAAGGUCUUACCG
Luc	26	SEQ. ID 0236	AAGUACCGAAAGGUCUUAC
Luc	27	SEQ. ID 0237	CGAAGUACCGAAAGGUCUU
Luc	28	SEQ. ID 0238	GACGAAGUACCGAAAGGUC
Luc	29	SEQ. ID 0239	UGGACGAAGUACCGAAAGG
Luc	30	SEQ. ID 0240	UGUGGACGAAGUACCGAAA
Luc	31	SEQ. ID 0241	UUUGUGGACGAAGUACCGA
Luc	32	SEQ. ID 0242	UGUUUGUGGACGAAGUACC
Luc	33	SEQ. ID 0243	UGUGUUUGUGGACGAAGUA
Luc	34	SEQ. ID 0244	GUUGUGUUUGUGGACGAAG
Luc	35	SEQ. ID 0245	GAGUUGUGUUUGUGGACGA
Luc	36	SEQ. ID 0246	AGGAGUUGUGUUUGUGGAC
Luc	37	SEQ. ID 0247	GGAGGAGUUGUGUUUGUGG
Luc	38	SEQ. ID 0248	GCGGAGGAGUUGUGUUUGU
Luc	39	SEQ. ID 0249	GCGCGGAGGAGUUGUGUUU
Luc	40	SEQ. ID 0250	UUGCGCGGAGGAGUUGUGU
Luc	41	SEQ. ID 0251	AGUUGCGCGGAGGAGUUGU
Luc	42	SEQ. ID 0252	AAAGUUGCGCGGAGGAGUU
Luc	43	SEQ. ID 0253	AAAAAGUUGCGCGGAGGAG
Luc	44	SEQ. ID 0254	CGAAAAAGUUGCGCGGAGG
Luc	45	SEQ. ID 0255	CGCGAAAAAGUUGCGCGGA
Luc	46	SEQ. ID 0256	ACCGCGAAAAAGUUGCGCG
Luc	47	SEQ. ID 0257	CAACCGCGAAAAAGUUGCG
Luc	48	SEQ. ID 0258	AACAACCGCGAAAAAGUUG
Luc	49	SEQ. ID 0259	GUAACAACCGCGAAAAAGU
Luc	50	SEQ. ID 0260	AAGUAACAACCGCGAAAAA
Luc	51	SEQ. ID 0261	UCAAGUAACAACCGCGAAA
Luc	52	SEQ. ID 0262	AGUCAAGUAACAACCGCGA
Luc	53	SEQ. ID 0263	CCAGUCAAGUAACAACCGC

Luc	54	SEQ. ID 0264	CGCCAGUCAAGUAACAACC
Luc	55	SEQ. ID 0265	GUCGCCAGUCAAGUAACAA
Luc	56	SEQ. ID 0266	ACGUCGCCAGUCAAGUAAC
Luc	57	SEQ. ID 0267	UUACGUCGCCAGUCAAGUA
Luc	58	SEQ. ID 0268	GAUUACGUCGCCAGUCAAG
Luc	59	SEQ. ID 0269	UGGAUUACGUCGCCAGUCA
Luc	60	SEQ. ID 0270	CGUGGAUUACGUCGCCAGU
Luc	61	SEQ. ID 0271	AUCGUGGAUUACGUCGCCA
Luc	62	SEQ. ID 0272	AGAUCGUGGAUUACGUCGC
Luc	63	SEQ. ID 0273	AGAGAUCGUGGAUUACGUC
Luc	64	SEQ. ID 0274	AAAGAGAUCGUGGAUUACG
Luc	65	SEQ. ID 0275	AAAAAGAGAUCGUGGAUUA
Luc	66	SEQ. ID 0276	GGAAAAAGAGAUCGUGGAU
Luc	67	SEQ. ID 0277	ACGGAAAAAGAGAUCGUGG
Luc	68	SEQ. ID 0278	UGACGGAAAAAGAGAUCGU
Luc	69	SEQ. ID 0279	GAUGACGGAAAAAGAGAUC
Luc	70	SEQ. ID 0280	ACGAUGACGGAAAAAGAGA
Luc	71	SEQ. ID 0281	AGACGAUGACGGAAAAAGA
Luc	72	SEQ. ID 0282	AAAGACGAUGACGGAAAAA
Luc	73	SEQ. ID 0283	GGAAAGACGAUGACGGAAA
Luc	74	SEQ. ID 0284	ACGGAAAGACGAUGACGGA
Luc	75	SEQ. ID 0285	GCACGGAAAGACGAUGACG
Luc	76	SEQ. ID 0286	GAGCACGGAAAGACGAUGA
Luc	77	SEQ. ID 0287	UGGAGCACGGAAAGACGAU
Luc	78	SEQ. ID 0288	UUUGGAGCACGGAAAGACG
Luc	79	SEQ. ID 0289	GUUUUGGAGCACGGAAAGA
Luc	80	SEQ. ID 0290	UUGUUUUGGAGCACGGAAA
Luc	81	SEQ. ID 0291	UGUUGUUUUGGAGCACGGA
Luc	82	SEQ. ID 0292	GUUGUUGUUUUGGAGCACG
Luc	83	SEQ. ID 0293	CCGUUGUUGUUUUGGAGCA
Luc	84	SEQ. ID 0294	CGCCGUUGUUGUUUUGGAG
Luc	85	SEQ. ID 0295	GCCGCCGUUGUUGUUUUGG
Luc	86	SEQ. ID 0296	CCGCCGCCGUUGUUGUUUU
Luc	87	SEQ. ID 0297	UCCCGCCGCCGUUGUUGUU
Luc	88	SEQ. ID 0298	CUUCCCGCCGCCGUUGUUG
Luc	89	SEQ. ID 0299	AACUUCCCGCCGCCGUUGU
Luc	90	SEQ. ID 0300	UGAACUUCCCGCCGCCGUU

Example II. Validation of the Algorithm using DBI, Luciferase, PLK, EGFR, and SEAP

The algorithm (Formula VIII) identified siRNAs for five genes, human DBI, firefly luciferase (fLuc), renilla luciferase (rLuc), human PLK, and human secreted alkaline phosphatase (SEAP). Four individual siRNAs were selected on the basis of their SMARTscores™ derived by analysis of their sequence using Formula VIII (all of the siRNAs would be selected with Formula IX as well) and analyzed for their ability to silence their targets' expression. In addition to the scoring, a BLAST search was conducted for each siRNA. To minimize the potential for off-target silencing effects, only those target sequences with more than three mismatches against unrelated sequences were selected. Semizarov, *et al, Specificity of short interfering RNA determined through gene expression signatures.* Proc. Natl. Acad. Sci. U.S.A. 2003, 100:6347. These duplexes were analyzed individually and in pools of 4 and compared with several siRNAs that were randomly selected. The functionality was measured a percentage of targeted gene knockdown as compared to controls. All siRNAs were transfected as described by the methods above at 100 nM concentration into HEK293 using Lipofectamine 2000. The level of the targeted gene expression was evaluated by B-DNA as described above and normalized to the non-specific control. **Figure 10** shows that the siRNAs selected by the algorithm disclosed herein were significantly more potent than randomly selected siRNAs. The algorithm increased the chances of identifying an F50 siRNA from 48% to 91%, and an F80 siRNA from 13% to 57%. In addition, pools of SMART siRNA silence the selected target better than randomly selected pools (see Figure 10F).

Example III. Validation of the Algorithm Using Genes Involved in Clathrin-Dependent Endocytosis.

Components of clathrin-mediated endocytosis pathway are key to modulating intracellular signaling and play important roles in disease. Chromosomal rearrangements that result in fusion transcripts between the Mixed-Lineage Leukemia gene (MLL) and CALM (Clathrin assembly lymphoid myeloid leukemia gene) are believed to play a role in leukemogenesis. Similarly, disruptions in Rab7 and Rab9, as well as HIP1 (Huntingtin-interacting protein), genes that are believed to be involved in endocytosis, are potentially responsible for ailments resulting in lipid storage, and neuronal diseases, respectively. For these reasons, siRNA directed

against clathrin and other genes involved in the clathrin-mediated endocytotic pathway are potentially important research and therapeutic tools.

siRNAs directed against genes involved in the clathrin-mediated endocytosis pathways were selected using Formula VIII. The targeted genes were clathrin heavy chain (CHC, accession # NM_004859), clathrin light chain A (CLCa, NM_001833), clathrin light chain B (CLCb, NM_001834), CALM (U45976), β2 subunit of AP-2 (β2, NM_001282), Eps15 (NM_001981), Eps15R (NM_021235), dynamin II (DYNII, NM_004945), Rab5a (BC001267), Rab5b (NM_002868), Rab5c (AF141304), and EEA.1 (XM_018197).

For each gene, four siRNAs duplexes with the highest scores were selected and a BLAST search was conducted for each of them using the Human EST database. In order to minimize the potential for off-target silencing effects, only those sequences with more than three mismatches against un-related sequences were used. All duplexes were synthesized at Dharmacon, Inc. as 21-mers with 3'-UU overhangs using a modified method of 2'-ACE chemistry Scaringe, *Advanced 5'-silyl-2'-orthoester approach to RNA oligonucleotide synthesis,* Methods Enzymol 2000, 317:3 and the antisense strand was chemically phosphorylated to insure maximized activity.

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, antibiotics and glutamine. siRNA duplexes were resuspended in 1X siRNA Universal buffer (Dharmacon, Inc.) to 20μM prior to transfection. HeLa cells in 12-well plates were transfected twice with 4μl of 20μM siRNA duplex in 3μl Lipofectamine 2000 reagent (Invitrogen, Carlsbad, California, USA) at 24-hour intervals. For the transfections in which 2 or 3 siRNA duplexes were included, the amount of each duplex was decreased, so that the total amount was the same as in transfections with single siRNAs. Cells were plated into normal culture medium 12 hours prior to experiments, and protein levels were measured 2 or 4 days after the first transfection.

Equal amounts of lysates were resolved by electrophoresis, blotted, and stained with the antibody specific to targeted protein, as well as antibodies specific to

unrelated proteins, PP1 phosphatase and Tsg101 (not shown). The cells were lysed in Triton X-100/glycerol solubilization buffer as described previously. Tebar, Bohlander, & Sorkin, *Clathrin Assembly Lymphoid Myeloid Leukemia (CALM) Protein: Localization in Endocytic-coated Pits, Interactions with Clathrin, and the Impact of Overexpression on Clathrin-mediated Traffic,* Mol. Biol. Cell Aug 1999, 10:2687. Cell lysates were electrophoresed, transferred to nitrocellulose membranes, and Western blotting was performed with several antibodies followed by detection using enhanced chemiluminescence system (Pierce, Inc). Several x-ray films were analyzed to determine the linear range of the chemiluminescence signals, and the quantifications were performed using densitometry and AlphaImager v5.5 software (Alpha Innotech Corporation). In experiments with Eps15R-targeted siRNAs, cell lysates were subjected to immunoprecipitation with Ab860, and Eps15R was detected in immunoprecipitates by Western blotting as described above.

The antibodies to assess the levels of each protein by Western blot were obtained from the following sources: monoclonal antibody to clathrin heavy chain (TD.1) was obtained from American Type Culture Collection (Rockville, MD, USA); polyclonal antibody to dynamin II was obtained from Affinity Bioreagents, Inc. (Golden, CO, USA); monoclonal antibodies to EEA.1 and Rab5a were purchased from BD Transduction Laboratories (Los Angeles, CA, USA); the monoclonal antibody to Tsg101 was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA); the monoclonal antibody to GFP was from ZYMED Laboratories Inc. (South San Francisco, CA, USA); the rabbit polyclonal antibodies Ab32 specific to α-adaptins and Ab20 to CALM were described previously Sorkin, *et al, Stoichiometric Interaction of the Epidermal Growth Factor Receptor with the Clathrin-associated Protein Complex AP-2, J. Biol. Chem.* Jan 1995, 270:619, the polyclonal antibodies to clathrin light chains A and B were kindly provided by Dr. F. Brodsky (UCSF); monoclonal antibodies to PP1 (BD Transduction Laboratories) and α-Actinin (Chemicon) were kindly provided by Dr. M. Dell'Acqua (University of Colorado); Eps15 Ab577 and Eps15R Ab860 were kindly provided by Dr. P.P. Di Fiore (European Cancer Institute).

Figure 11 demonstrates the *in vivo* functionality of 48 individual siRNAs, selected using Formula VIII (most of them will meet the criteria incorporated by Formula IX as well) targeting 12 genes. Various cell lines were transfected with siRNA duplexes (***Dup1-4***) or pools of siRNA duplexes (Pool), and the cells were lysed 3 days after transfection with the exception of CALM (2 days) and β2 (4 days).

Note a β1-adaptin band (part of AP-1 Golgi adaptor complex) that runs slightly slower than β2 adaptin. CALM has two splice variants, 66 and 72 kD. The full-length Eps15R (a doublet of ~130 kD) and several truncated spliced forms of ~ 100 kD and ~70 kD were detected in Eps15R immunoprecipitates (shown by arrows). The cells were lysed 3 days after transfection. Equal amounts of lysates were resolved by electrophoresis and blotted with the antibody specific to a targeted protein (GFP antibody for YFP fusion proteins) and the antibody specific to unrelated proteins PP1 phosphatase or α-actinin, and TSG101. The amount of protein in each specific band was normalized to the amount of non-specific proteins in each lane of the gel. Nearly all of them appear to be functional, which establishes that Formula VIII and IX can be used to predict siRNAs' functionality in general in a genome wide manner.

To generate the fusion of yellow fluorescent protein (YFP) with Rab5b or Rab5c (YFP-Rab5b or YFP-Rab5c), a DNA fragment encoding the full-length human Rab5b or Rab5c was obtained by PCR using *Pfu* polymerase (Stratagene) with a *Sac*I restriction site introduced into the 5' end and a *Kpn*I site into the 3' end and cloned into pEYFP-C1 vector (CLONTECH, Palo Alto, CA, USA). GFP-CALM and YFP-Rab5a were described previously Tebar, Bohlander, & Sorkin, *Clathrin Assembly Lymphoid Myeloid Leukemia (CALM) Protein: Localization in Endocytic-coated Pits, Interactions with Clathrin, and the Impact of Overexpression on Clathrin-mediated Traffic*, Mol. Biol. Cell Aug 1999, 10:2687.

Example III. Validation of the Algorithm Using Eg5, GADPH, ATE1, MEK2, MEK1, QB, LaminA/C, c-myc, human cyclophilin, and mouse cyclophilin.

A number of genes have been identified as playing potentially important roles in disease etiology. Expression profiles of normal and diseased kidneys has implicated Edg5 in immunoglobulin A neuropathy, a common renal glomerular disease. Mycl, MEK1/2 and other related kinases have been associated with one or more cancers, while lamins have been implicated in muscular dystrophy and other diseases. For these reasons, siRNA directed against the genes encoding these classes of molecules would be important research and therapeutic tools.

Figure 12 illustrates four siRNAs targeting 10 different genes (**Table V** for sequence and accession number information) that were selected according to the Formula VIII and assayed as individuals and pools in HEK293 cells. The level of siRNA induced silencing was measured using the B-DNA assay. These studies demonstrated that thirty-six out of the forty individual SMART-selected siRNA tested are functional (90%) and all 10 pools are fully functional.

Example V. Validation of the Algorithm Using Bcl2

Bcl-2 is a ~25kD, 205-239 amino acid, anti-apoptotic protein that contains considerable homology with other members of the BCL family including BCLX, MCL1, BAX, BAD, and BIK. The protein exists in at least two forms (Bcl2a, which has a hydrophobic tail for membrane anchorage, and Bcl2b, which lacks the hydrophobic tail) and is predominantly localized to the mitochondrial membrane. While Bcl2 expression is widely distributed, particular interest has focused on the expression of this molecule in B and T cells. Bcl2 expression is down-regulated in normal germinal center B cells yet in a high percentage of follicular lymphomas, Bcl2 expression has been observed to be elevated. Cytological studies have identified a common translocation ((14;18)(q32;q32)) amongst a high percentage (>70%) of these lymphomas. This genetic lesion places the Bcl2 gene in juxtaposition to immunoglobulin heavy chain gene (IgH) encoding sequences and is believed to enforce inappropriate levels of gene expression, and resistance to programmed cell death in the follicle center B cells. In other cases, hypomethylation of the Bcl2 promoter leads to enhanced expression and again, inhibition of apoptosis. In addition to cancer, dysregulated expression of Bcl-2 has been correlated with multiple sclerosis and various neurological diseases.

The correlation between Bcl-2 translocation and cancer makes this gene an attractive target for RNAi. Identification of siRNA directed against the bcl2 transcript (or Bcl2-IgH fusions) would further our understanding Bcl2 gene function and possibly provide a future therapeutic agent to battle diseases that result from altered expression or function of this gene.

In Silico Identification of Functional siRNA

To identify functional and hyperfunctional siRNA against the Bcl2 gene, the sequence for Bcl-2 was downloaded from the NCBI Unigene database and analyzed using the Formula VIII algorithm. As a result of these procedures, both the sequence and SMARTscores™ of the Bcl2 siRNA were obtained and ranked according to their functionality. Subsequently, these sequences were BLAST'ed (database) to insure that the selected sequences were specific and contained minimal overlap with unrealated genes. The SMARTscores™ for the top 10 Bcl-2 siRNA are identified in **Figure 13**.

In Vivo Testing of Bcl-2 SiRNA

Bcl-2 siRNAs having the top ten SMARTscores™ were selected and tested in a functional assay to determine silencing efficiency. To accomplish this, each of the ten duplexes were synthesized using 2'-O-ACE chemistry and transfected at 100nM concentrations into cells. Twenty-four hours later assays were performed on cell extracts to assess the degree of target silencing. Controls used in these experiments included mock transfected cells, and cells that were transfected with a non-specific siRNA duplex.

The results of these experiments are presented below (and in **Figure 14**) and show that all ten of the selected siRNA induce 80% or better silencing of the Bcl2 message at 100nM concentrations. These data verify that the algorithm successfully identified functional Bcl2 siRNA and provide a set of functional agents that can be used in experimental and therapeutic environments.

siRNA 1	GGGAGAUAGUGAUGAAGUA	SEQ. ID NO. 301
siRNA 2	GAAGUACAUCCAUUAUAAG	SEQ. ID NO. 302
siRNA 3	GUACGACAACCGGGAGAUA	SEQ. ID NO. 303
siRNA 4	AGAUAGUGAUGAAGUACAU	SEQ. ID NO. 304
siRNA 5	UGAAGACUCUGCUCAGUUU	SEQ. ID NO. 305
siRNA 6	GCAUGCGGCCUCUGUUUGA	SEQ. ID NO. 306
siRNA 7	UGCGGCCUCUGUUUGAUUU	SEQ. ID NO. 307
siRNA 8	GAGAUAGUGAUGAAGUACA	SEQ. ID NO. 308
siRNA 9	GGAGAUAGUGAUGAAGUAC	SEQ. ID NO. 309
siRNA 10	GAAGACUCUGCUCAGUUUG	SEQ. ID NO. 310

Bcl2 siRNA: Sense Strand, 5'→3'

Example VI. Sequences Selected by the Algorithm

Sequences of the siRNAs selected using Formulas (Algorithms) VIII and IX with their corresponding ranking, which have been evaluated for the silencing activity *in vivo* in the present study (Formula VIII and IX, respectively).

TABLE V

Gene Name	Accession Number	SEQ. ID NO.	FTIISeqTence	Formula VIII	Formula IX
CLTC	NM_004859	SEQ. ID NO. 0301	GAAAGAATCTGTAGAGAAA	76	94.2
CLTC	NM_004859	SEQ. ID NO. 0302	GCAATGAGCTGTTTGAAGA	65	39.9
CLTC	NM_004859	SEQ. ID NO. 0303	TGACAAAGGTGGATAAATT	57	38.2
CLTC	NM_004859	SEQ. ID NO. 0304	GGAAATGGATCTCTTTGAA	54	49.4
CLTA	NM_001833	SEQ. ID NO. 0305	GGAAAGTAATGGTCCAACA	22	55.5
CLTA	NM_001833	SEQ. ID NO. 0306	AGACAGTTATGCAGCTATT	4	22.9
CLTA	NM_001833	SEQ. ID NO. 0307	CCAATTCTCGGAAGCAAGA	1	17
CLTA	NM_001833	SEQ. ID NO. 0308	GAAAGTAATGGTCCAACAG	-1	-13
CLTB	NM_001834	SEQ. ID NO. 0309	GCGCCAGAGTGAACAAGTA	17	57.5
CLTB	NM_001834	SEQ. ID NO. 0310	GAAGGTGGCCCAGCTATGT	15	-8.6
CLTB	NM_001834	SEQ. ID NO. 0311	GGAACCAGCGCCAGAGTGA	13	40.5
CLTB	NM_001834	SEQ. ID NO. 0312	GAGCGAGATTGCAGGCATA	20	61.7
CALM	U45976	SEQ. ID NO. 0313	GTTAGTATCTGATGACTTG	36	-34.6
CALM	U45976	SEQ. ID NO. 0314	GAAATGGAACCACTAAGAA	33	46.1
CALM	U45976	SEQ. ID NO. 0315	GGAAATGGAACCACTAAGA	30	61.2
CALM	U45976	SEQ. ID NO. 0316	CAACTACACTTTCCAATGC	28	6.8
EPS15	NM_001981	SEQ. ID NO. 0317	CCACCAAGATTTCATGATA	48	25.2

EPS15	NM_001981	SEQ. ID NO. 0318	GATCGGAACTCCAACAAGA	43	49.3
EPS15	NM_001981	SEQ. ID NO. 0319	AAACGGAGCTACAGATTAT	39	11.5
EPS15	NM_001981	SEQ. ID NO. 0320	CCACACAGCATTCTTGTAA	33	-23.6
EPS15R	NM_021235	SEQ. ID NO. 0321	GAAGTTACCTTGAGCAATC	48	33
EPS15R	NM_021235	SEQ. ID NO. 0322	GGACTTGGCCGATCCAGAA	27	33
EPS15R	NM_021235	SEQ. ID NO. 0323	GCACTTGGATCGAGATGAG	20	1.3
EPS15R	NM_021235	SEQ. ID NO. 0324	CAAAGACCAATTCGCGTTA	17	27.7
DNM2	NM_004945	SEQ. ID NO. 0325	CCGAATCAATCGCATCTTC	6	-29.6
DNM2	NM_004945	SEQ. ID NO. 0326	GACATGATCCTGCAGTTCA	5	-14
DNM2	NM_004945	SEQ. ID NO. 0327	GAGCGAATCGTCACCACTT	5	24
DNM2	NM_004945	SEQ. ID NO. 0328	CCTCCGAGCTGGCGTCTAC	-4	-63.6
ARF6	AF93885	SEQ. ID NO. 0329	TCACATGGTTAACCTCTAA	27	-21.1
ARF6	AF93885	SEQ. ID NO. 0330	GATGAGGGACGCCATAATC	7	-38.4
ARF6	AF93885	SEQ. ID NO. 0331	CCTCTAACTACAAATCTTA	4	16.9
ARF6	AF93885	SEQ. ID NO. 0332	GGAAGGTGCTATCCAAAAT	4	11.5
RAB5A	BC001267	SEQ. ID NO. 0333	GCAAGCAAGTCCTAACATT	40	25.1
RAB5A	BC001267	SEQ. ID NO. 0334	GGAAGAGGAGTAGACCTTA	17	50.1
RAB5A	BC001267	SEQ. ID NO. 0335	AGGAATCAGTGTTGTAGTA	16	11.5
RAB5A	BC001267	SEQ. ID NO. 0336	GAAGAGGAGTAGACCTTAC	12	7
RAB5B	NM_002868	SEQ. ID NO. 0337	GAAAGTCAAGCCTGGTATT	14	18.1
RAB5B	NM_002868	SEQ. ID NO. 0338	AAAGTCAAGCCTGGTATTA	6	-17.8
RAB5B	NM_002868	SEQ. ID NO. 0339	GCTATGAACGTGAATGATC	3	-21.1
RAB5B	NM_002868	SEQ. ID NO. 0340	CAAGCCTGGTATTACGTTT	-7	-37.5
RAB5C	AF141304	SEQ. ID NO. 0341	GGAACAAGATCTGTCAATT	38	51.9
RAB5C	AF141304	SEQ. ID NO. 0342	GCAATGAACGTGAACGAAA	29	43.7
RAB5C	AF141304	SEQ. ID NO. 0343	CAATGAACGTGAACGAAAT	18	43.3
RAB5C	AF141304	SEQ. ID NO. 0344	GGACAGGAGCGGTATCACA	6	18.2
EEA1	XM_018197	SEQ. ID NO. 0345	AGACAGAGCTTGAGAATAA	67	64.1
EEA1	XM_018197	SEQ. ID NO. 0346	GAGAAGATCTTTATGCAAA	60	48.7
EEA1	XM_018197	SEQ. ID NO. 0347	GAAGAGAAATCAGCAGATA	58	45.7
EEA1	XM_018197	SEQ. ID NO. 0348	GCAAGTAACTCAACTAACA	56	72.3
AP2B1	NM_001282	SEQ. ID NO. 0349	GAGCTAATCTGCCACATTG	49	-12.4
AP2B1	NM_001282	SEQ. ID NO. 0350	GCAGATGAGTTACTAGAAA	44	48.9
AP2B1	NM_001282	SEQ. ID NO. 0351	CAACTTAATTGTCCAGAAA	41	28.2
AP2B1	NM_001282	SEQ. ID NO. 0352	CAACACAGGATTCTGATAA	33	-5.8
PLK	NM_005030	SEQ. ID NO. 0353	AGATTGTGCCTAAGTCTCT	-35	-3.4
PLK	NM_005030	SEQ. ID NO. 0354	ATGAAGATCTGGAGGTGAA	0	-4.3
PLK	NM_005030	SEQ. ID NO. 0355	TTTGAGACTTCTTGCCTAA	-5	-27.7
PLK	NM_005030	SEQ. ID NO. 0356	AGATCACCCTCCTTAAATA	15	72.3
GAPDH	NM_002046	SEQ. ID NO. 0357	CAACGGATTTGGTCGTATT	27	-2.8

GAPDH	NM_002046	SEQ. ID NO. 0358	GAAATCCCATCACCATCTT	24	3.9
GAPDH	NM_002046	SEQ. ID NO. 0359	GACCTCAACTACATGGTTT	22	-22.9
GAPDH	NM_002046	SEQ. ID NO. 0360	TGGTTTACATGTTCCAATA	9	9.8
c-Myc		SEQ. ID NO. 0361	GAAGAAATCGATGTTGTTT	31	-11.7
c-Myc		SEQ. ID NO. 0362	ACACAAACTTGAACAGCTA	22	51.3
c-Myc		SEQ. ID NO. 0363	GGAAGAAATCGATGTTGTT	18	26
c-Myc		SEQ. ID NO. 0364	GAAACGACGAGAACAGTTG	18	-8.9
MAP2K1	NM_002755	SEQ. ID NO. 0365	GCACATGGATGGAGGTTCT	26	16
MAP2K1	NM_002755	SEQ. ID NO. 0366	GCAGAGAGAGCAGATTTGA	16	0.4
MAP2K1	NM_002755	SEQ. ID NO. 0367	GAGGTTCTCTGGATCAAGT	14	15.5
MAP2K1	NM_002755	SEQ. ID NO. 0368	GAGCAGATTTGAAGCAACT	14	18.5
MAP2K2	NM_030662	SEQ. ID NO. 0369	CAAAGACGATGACTTCGAA	37	26.4
MAP2K2	NM_030662	SEQ. ID NO. 0370	GATCAGCATTTGCATGGAA	24	-0.7
MAP2K2	NM_030662	SEQ. ID NO. 0371	TCCAGGAGTTTGTCAATAA	17	-4.5
MAP2K2	NM_030662	SEQ. ID NO. 0372	GGAAGCTGATCCACCTTGA	16	59.2
KNSL1(EG5)	NM_004523	SEQ. ID NO. 0373	GCAGAAATCTAAGGATATA	53	35.8
KNSL1(EG5)	NM_004523	SEQ. ID NO. 0374	CAACAAGGATGAAGTCTAT	50	18.3
KNSL1(EG5)	NM_004523	SEQ. ID NO. 0375	CAGCAGAAATCTAAGGATA	41	32.7
KNSL1(EG5)	NM_004523	SEQ. ID NO. 0376	CTAGATGGCTTTCTCAGTA	39	3.9
CyclophilinA_	NM_021130	SEQ. ID NO. 0377	AGACAAGGTCCCAAAGACA	-16	58.1
CyclophilinA_	NM_021130	SEQ. ID NO. 0378	GGAATGGCAAGACCAGCAA	-6	36
CyclophilinA_	NM_021130	SEQ. ID NO. 0379	AGAATTATTCCAGGGTTTA	-3	16.1
CyclophilinA_	NM_021130	SEQ. ID NO. 0380	GCAGACAAGGTCCCAAAGA	8	8.9
LAMIN A/C	NM_170707	SEQ. ID NO. 0381	AGAAGCAGCTTCAGGATGA	31	38.8
LAMIN A/C	NM_170707	SEQ. ID NO. 0382	GAGCTTGACTTCCAGAAGA	33	22.4
LAMIN A/C	NM_170707	SEQ. ID NO. 0383	CCACCGAAGTTCACCCTAA	21	27.5
LAMIN A/C	NM_170707	SEQ. ID NO. 0384	GAGAAGAGCTCCTCCATCA	55	30.1
CyclophilinB	M60857	SEQ. ID NO. 0385	GAAAGAGCATCTACGGTGA	41	83.9
CyclophilinB	M60857	SEQ. ID NO. 0386	GAAAGGATTTGGCTACAAA	53	59.1
CyclophilinB	M60857	SEQ. ID NO. 0387	ACAGCAAATTCCATCGTGT	-20	28.8
CyclophilinB	M60857	SEQ. ID NO. 0388	GGAAAGACTGTTCCAAAAA	2	27
DBI1	NM_020548	SEQ. ID NO. 0389	CAACACGCCTCATCCTCTA	27	-7.6
DBI2	NM_020548	SEQ. ID NO. 0390	CATGAAAGCTTACATCAAC	25	-30.8
DBI3	NM_020548	SEQ. ID NO. 0391	AAGATGCCATGAAAGCTTA	17	22
DBI4	NM_020548	SEQ. ID NO. 0392	GCACATACCGCCTGAGTCT	15	3.9
rLUC1		SEQ. ID NO. 0393	GATCAAATCTGAAGAAGGA	57	49.2
rLUC2		SEQ. ID NO. 0394	GCCAAGAAGTTTCCTAATA	50	13.7
rLUC3		SEQ. ID NO. 0395	CAGCATATCTTGAACCATT	41	-2.2
rLUC4		SEQ. ID NO. 0396	GAACAAAGGAAACGGATGA	39	29.2
SeAP1	NM_031313	SEQ. ID NO. 0397	CGGAAACGGTCCAGGCTAT	6	26.9

SeAP2	NM_031313	SEQ. ID NO. 0398	GCTTCGAGCAGACATGATA	4	-11.2
SeAP3	NM_031313	SEQ. ID NO. 0399	CCTACACGGTCCTCCTATA	4	4.9
SeAP4	NM_031313	SEQ. ID NO. 0400	GCCAAGAACCTCATCATCT	1	-9.9
fLUC1		SEQ. ID NO. 0401	GATATGGGCTGAATACAAA	54	40.4
fLUC2		SEQ. ID NO. 0402	GCACTCTGATTGACAAATA	47	54.7
fLUC3		SEQ. ID NO. 0403	TGAAGTCTCTGATTAAGTA	46	34.5
fLUC4		SEQ. ID NO. 0404	TCAGAGAGATCCTCATAAA	40	11.4
mCyclo_1	NM_008907	SEQ. ID NO. 0405	GCAAGAAGATCACCATTTC	52	46.4
mCyclo_2	NM_008907	SEQ. ID NO. 0406	GAGAGAAATTTGAGGATGA	36	70.7
mCyclo_3	NM_008907	SEQ. ID NO. 0407	GAAAGGATTTGGCTATAAG	35	-1.5
mCyclo_4	NM_008907	SEQ. ID NO. 0408	GAAAGAAGGCATGAACATT	27	10.3
BCL2_1	NM_000633	SEQ. ID NO. 0409	GGGAGATAGTGATGAAGTA	21	72
BCL2_2	NM_000633	SEQ. ID NO. 0410	GAAGTACATCCATTATAAG	1	3.3
BCL2_3	NM_000633	SEQ. ID NO. 0411	GTACGACAACCGGGAGATA	1	35.9
BCL2_4	NM_000633	SEQ. ID NO. 0412	AGATAGTGATGAAGTACAT	-12	22.1
BCL2_5	NM_000633	SEQ. ID NO. 0413	TGAAGACTCTGCTCAGTTT	36	19.1
BCL2_6	NM_000633	SEQ. ID NO. 0414	GCATGCGGCCTCTGTTTGA	5	-9.7
QB1	NM_003365.1	SEQ. ID NO. 0415	GCACACAGCUUACUACAUC	52	-4.8
QB2	NM_003365.1	SEQ. ID NO. 0416	GAAAUGCCCUGGUAUCUCA	49	22.1
QB3	NM_003365.1	SEQ. ID NO. 0417	GAAGGAACGUGAUGUGAUC	34	22.9
QB4	NM_003365.1	SEQ. ID NO. 0418	GCACUACUCCUGUGUGUGA	28	20.4
ATE1-1	NM_007041	SEQ. ID NO. 0419	GAACCCAGCUGGAGAACUU	45	15.5
ATE1-2	NM_007041	SEQ. ID NO. 0420	GAUAUACAGUGUGAUCUUA	40	12.2
ATE1-3	NM_007041	SEQ. ID NO. 0421	GUACUACGAUCCUGAUUAU	37	32.9
ATE1-4	NM_007041	SEQ. ID NO. 0422	GUGCCGACCUUUACAAUUU	35	18.2
EGFR-1	NM_005228	SEQ. ID NO. 0423	GAAGGAAACTGAATTCAAA	68	79.4
EGFR-1	NM_005228	SEQ. ID NO. 0424	GGAAATATGTACTACGAAA	49	49.5
EGFR-1	NM_005228	SEQ. ID NO. 0425	CCACAAAGCAGTGAATTTA	41	7.6
EGFR-1	NM_005228	SEQ. ID NO. 0426	GTAACAAGCTCACGCAGTT	40	25.9

Example VII. Genome-Wide Application of the Algorithm

The examples described above demonstrate that the algorithm(s) can successfully identify functional siRNA and that these duplexes can be used to induce the desirable phenotype of transcriptional knockdown or knockout. Each gene or family of genes in each organism plays an important role in maintaining physiological homeostasis and the algorithm can be used to develop functional, highly functional, or hyperfunctional siRNA to each gene. To accomplish this for the human genome, the entire online ncbi refseq database was accessed through Entrez (efetch). The database

was processed through Formula VIII. For each gene the top 80 –100 scores for siRNAs were obtained and BLAST'ed to insure that the selected sequences are specific in targeting the gene of choice. These sequences are provided on the enclosed CDs in electronic form. Accordingly, Applicants hereby incorporate by reference the material submitted herewith, in duplicate on the compact disks labeled COPY 1 – TABLES PART, DISK 1/1, TABLES 12 –15, Filed with RO/US under PCT AI sec. 801(a), Operating System: MS-Windows, COPY 2 – TABLES PART, DISK 1/1, TABLES 12 –15, Filed with RO/US under PCT AI sec. 801(a), Operating System: MS-Windows, COPY 3 – TABLES PART, DISK 1/1, TABLES 12 –15, Filed with RO/US under PCT AI sec. 801(a), Operating System: MS-Windows,; which copies are identical, in files entitled Table_12.txt, date of creation June 26, 2003, with a size of 31,045 kb; Table_13.txt, date of creation November 13, 2003, with a size of 78,451 kb; Table_14.txt, date of creation November 13, 2003, with a size of 454 kb; and Table_15.txt date of creation November 13, 2003, with a size of 1,690 kb.

With respect to the disks, there are four tables on each disk copy in text format: Tables XII –XV. Table XII, which is located in a file entitled Table_12.txt, provides a list of the 80-100 sequences for each target, identified by Formula VIII as having the highest relative SMARTscores™ for the target analyzed. Table XIII, which is located in a file entitled Table_13.txt, provides the SMARTscores™, and for each gene, a pool pick of up to four sequences is denoted. (The denotation of "1" in Table XIII means that it is a pool pick.) These pool pick sequences represent the most functional siRNAs for the corresponding target. Any 1, 2, 3, or 4 of the pool pick sequences could be used for gene silencing. Further, sequences that are not denoted as pool pick sequences, but that are included on the compact disks may also be used for gene silencing either alone or in combination with other sequences. However, their individual relative functionality would be less than that of a pool pick sequence. Table XIV, which is located in a file entitled Table_14.txt, provides an identification of genes by accession number, and Table XV, which is located in a file entitled Table_15.txt, provides a short name for the genes identified on the disk. The information contained on the disks is part of this patent application and are incorporated into the specification by reference. One may use these tables in order to identify functional siRNAs for the gene provided therein, by simply looking for the

gene of interest and an siRNA that is listed as functional. Preferably, one would select one or more of the siRNA that most optimized for the target of interest and is denoted as a pool pick.

Table XII: siRNA selected by Formula VIII

See data submitted herewith on a CD-ROM in accordance with PCT Administrative Instructions Section 801(a)

Table XIII: SMARTscores™

See data submitted herewith on a CD-ROM in accordance with PCT Administrative Instructions Section 801(a)

Table XIV: Identification of Targets

See data submitted herewith on a CD-ROM in accordance with PCT Administrative Instructions Section 801(a)

Table XV: Description of Targts

See data submitted herewith on a CD-ROM in accordance with PCT Administrative Instructions Section 801(a)

Many of the genes to which the described siRNA are directed play critical roles in disease etiology. For this reason, the siRNA listed in the accompanying compact disk may potentially act as therapeutic agents. A number of prophetic examples follow and should be understood in view of the siRNA that are identified on the accompanying CD. To isolate these siRNA, the appropriate message sequence for each gene is analyzed using one of the before mentioned formulas (preferably formula VIII) to identify potential siRNA targets. Subsequently these targets are BLAST'ed to eliminate homology with potentially off-targets.

The list of potential disease targets is extensive. For instance, over-expression of Bcl10 has been implicated in the development of MALT lymphoma (mucosa associated lymphoid tissue lymphoma) and thus, functional, highly functional, or hyperfunctional siRNA directed against that gene (*e.g.* SEQ. ID NO. 0427: GGAAACCUCUCAUUGCUAA; SEQ. ID NO. 0428:

GAAAGAACCUUGCCGAUCA; SEQ. ID NO. 0429:
GGAAAUACAUCAGAGCUUA, or SEQ. ID NO. 0430:
GAAAGUAUGUGUCUUAAGU) may contribute to treatment of this disorder.

In another example, studies have shown that molecules that inhibit glutamine:fructose-6-phosphate aminotransferase (GFA) may act to limit the symptoms suffered by Type II diabetics. Thus, functional, highly functional, or hyperfunctional siRNA directed against GFA (also known as GFPT1: siRNA = SEQ. ID NO. 0433 UGAAACGGCUGCCUGAUUU; SEQ. ID NO. 0434
GAAGUUACCUCUUACAUUU; SEQ. ID NO. 0435
GUACGAAACUGUAUGAUUA; SEQ. ID NO. 0436
GGACGAGGCUAUCAUUAUG) may contribute to treatment of this disorder.

In another example, the von Hippel-Lindau (VHL) tumor suppressor has been observed to be inactivated at a high frequency in sporadic clear cell renal cell carcinoma (RCC) and RCCs associated with VHL disease. The VHL tumor suppressor targets hypoxia-inducible factor-1 alpha (HIF-1 alpha), a transcription factor that can induce vascular endothelial growth factor (VEGF) expression, for ubiquitination and degradation. Inactivation of VHL can lead to increased levels of HIF-1 alpha, and subsequent VEGF over expression. Such over expression of VEGF has been used to explain the increased (and possibly necessary) vascularity observed in RCC. Thus, functional, highly functional, or hyperfunctional siRNAs directed against either HIF-1 alpha (SEQ. ID NO. 0437 GAAGGAACCUGAUGCUUUA; SEQ. ID NO. 0438 GCAUAUAUCUAGAAGGUAU; SEQ. ID NO. 0439
GAACAAAUACAUGGGAUUA; SEQ. ID NO. 0440
GGACACAGAUUUAGACUUG) or VEGF (SEQ. ID NO. 0441
GAACGUACUUGCAGAUGUG; SEQ. ID NO. 0442
GAGAAAGCAUUUGUUUGUA; SEQ. ID NO. 0443
GGAGAAAGCAUUUGUUUGU; SEQ. ID NO. 0444
CGAGGCAGCUUGAGUUAAA) may be useful in the treatment of renal cell carcinoma.

In another example, gene expression of platelet derived growth factor A and B (PDGF-A and PDGF-B) has been observed to be increased 22- and 6-fold,

respectively, in renal tissues taken from patients with diabetic nephropathy as compared with controls. These findings suggest that over expression of PDGF A and B may play a role in the development of the progressive fibrosis that characterizes human diabetic kidney disease. Thus, functional, highly functional, or hyperfunctional siRNAs directed against either PDGF A

(SEQ. ID NO. 0445: GGUAAGAUAUUGUGCUUUA;
SEQ. ID NO. 0446: CCGCAAAUAUGCAGAAUUA;
SEQ. ID NO. 0447: GGAUGUACAUGGCGUGUUA;
SEQ. ID NO. 0448: GGUGAAGUUUGUAUGUUUA) or

PDGF B

(SEQ. ID NO. 0449: CCGAGGAGCUUUAUGAGAU;
SEQ. ID NO. 0450: GCUCCGCGCUUUCCGAUUU;
SEQ. ID NO. 0451 GAGCAGGAAUGGUGAGAUG;
SEQ. ID NO. 0452: GAACUUGGGAUAAGAGUGU;
SEQ. ID NO. 0453 CCGAGGAGCUUUAUGAGAU;
SEQ. ID NO. 0454 UUUAUGAGAUGCUGAGUGA) may be useful in the treatment of this form of kidney disorder.

In another example, a strong correlation exists between the over-expression of glucose transporters (*e.g.* GLUT12) and cancer cells. It is predicted that cells undergoing uncontrolled cell growth up-regulate GLUT molecules so that they can cope with the heightened energy needs associated with increased rates of proliferation and metastasis. Thus, siRNA-based therapies that target the molecules such as GLUT1 (also known as SLC2A1: siRNA=

SEQ. ID NO.: 0455 GCAAUGAUGUCCAGAAGAA;
SEQ. ID NO.: 0456 GAAGAAUAUUCAGGACUUA;
SEQ. ID NO.: 0457 GAAGAGAGUCGGCAGAUGA;
SEQ. ID NO.: 0458 CCAAGAGUGUGCUAAAGAA)

GLUT12 (also known as SLCA12: siRNA =

SEQ. ID NO. 0459: GAGACACUCUGAAAUGAUA;
SEQ. ID NO. 0460: GAAAUGAUGUGGAUAAGAG;
SEQ. ID NO. 0461: GAUCAAAUCCUCCCUGAAA;

SEQ. ID NO. 0462: UGAAUGAGCUGAUGAUUGU) and other related transporters, may be of value in treating a multitude of malignancies.

The siRNA sequences listed above are presented in a 5'→ 3' sense strand direction. In addition, siRNA directed against the targets listed above as well as those directed against other targets and listed in the accompanying compact disk may be useful as therapeutic agents.

Example VIII. Evidence for the Benefits of Pooling

Evidence for the benefits of pooling have been demonstrated using the reporter gene, luciferase. Ninety siRNA duplexes were synthesized using Dharmacon proprietary ACE® chemistry against one of the standard reporter genes: firefly luciferase. The duplexes were designed to start two base pairs apart and to cover approximately 180 base pairs of the luciferase gene (see sequences in **Table III**). Subsequently, the siRNA duplexes were co-transfected with a luciferase expression reporter plasmid into HEK293 cells using standard transfection protocols and luciferase activity was assayed at 24 and 48 hours.

Transfection of individual siRNAs showed standard distribution of inhibitory effect. Some duplexes were active, while others were not. **Figure 15** represents a typical screen of ninety siRNA duplexes (SEQ. ID NO. 0032- 0120) positioned two base pairs apart. As the figure suggests, the functionality of the siRNA duplex is determined more by a particular sequence of the oligonucleotide than by the relative oligonucleotide position within a gene or excessively sensitive part of the mRNA, which is important for traditional anti-sense technology.

When two continuous oligonucleotides were pooled together, a significant increase in gene silencing activity was observed. **(See Figure 16)** A gradual increase in efficacy and the frequency of pools functionality was observed when the number of siRNAs increased to 3 and 4. **(Figures 16, 17)**. Further, the relative positioning of the oligonucleotides within a pool did not determine whether a particular pool was functional **(see Figure 18, in which 100% of pools of oligonucleotides distanced by 2, 10 and 20 base pairs were functional).**

However, relative positioning may nonetheless have an impact. An increased functionality may exist when the siRNA are positioned continuously head to toe (5' end of one directly adjacent to the 3' end of the others).

Additionally, siRNA pools that were tested performed at least as well as the best oligonucleotide in the pool, under the experimental conditions whose results are depicted in **Figure 19**. Moreover, when previously identified non-functional and marginally (semi) functional siRNA duplexes were pooled together in groups of five at a time, a significant functional cooperative action was observed. (See **Figure 20**) In fact, pools of semi-active oligonucleotides were 5 to 25 times more functional than the most potent oligonucleotide in the pool. Therefore, pooling several siRNA duplexes together does not interfere with the functionality of the most potent siRNAs within a pool, and pooling provides an unexpected significant increase in overall functionality

Example IX. Pooling Across Species

Experiments were performed on the following genes: β-galactosidase, Renilla luciferase, and Secreted alkaline phosphatase, which demonstrates the benefits of pooling. (see **Figure 21**) Approximately 50% of individual siRNAs designed to silence the above-specified genes were functional, while 100% of the pools that contain the same siRNA duplexes were functional.

Example X. Highly Functional siRNA

Pools of five siRNAs in which each two siRNAs overlap to 10-90% resulted in 98% functional entities (>80% silencing). Pools of siRNAs distributed throughout the mRNA that were evenly spaced, covering an approximate 20 – 2000 base pair range, were also functional. When the pools of siRNA were positioned continuously head to tail relative to mRNA sequences and mimicked the natural products of Dicer cleaved long double stranded RNA, 98% of the pools evidenced highly functional activity (>95% silencing).

Example XI. Human cyclophyline

Table III above lists the siRNA sequences for the human cyclophyline protein. A particularly functional siRNA may be selected by applying these sequences to any of Formula I to VII above.

Alternatively, one could pool 2, 3, 4, 5 or more of these sequences to create a kit for silencing a gene. Preferably, within the kit there would be at least one sequence that has a relatively high predicted functionality when any of Formulas I - VII is applied.

Example XII. Sample Pools of siRNAs and Their Application to Human Disease

The genetic basis behind human disease is well documented and siRNA may be used as both research or diagnostic tools and therapeutic agents; either individually or in pools. Genes involved in signal transduction, the immune response, apoptosis, DNA repair, cell cycle control, and a variety of other physiological functions have clinical relevance and therapeutic agents that can modulate expression of these genes may alleviate some or all of the associated symptoms. In some instances, these genes can be described as a member of a family or class of genes and siRNA (randomly, conventionally, or rationally designed) can be directed against one or multiple members of the family to induce a desired result.

To identify rationally designed siRNA to each gene, the sequence was analyzed using Formula VIII to identify a SMARTpool containing the functional sequences. To confirm the activity of these sequences, the siRNA are introduced into a cell type of choice (*e.g.* HeLa cells, HEK293 cells) and the levels of the appropriate message are analyzed using one of several art proven techniques. SiRNA having heightened levels of potency can be identified by testing each of the before mentioned duplexes at increasingly limiting concentrations. Similarly, siRNA having increased levels of longevity can be identified by introducing each duplex into cells and testing functionality at 24, 48, 72, 96, 120, 144, 168, and 192 hours after transfection. Agents that induce >95% silencing at sub-nanomolar concentrations and/or induce functional levels of silencing for >96 hours are considered hyperfunctional.

The following are non-limiting examples of families of proteins to which siRNA described in this document are targeted against:

Transporters, Pumps, and Channels

Transporters, pumps, and channels represent one class of genes that are attractive targets for siRNAs. One major class of transporter molecules are the ATP-binding cassette (ABC) transporters. To date, nearly 50 human ABC-transporter genes have been characterized and have been shown to be involved in a variety of physiological functions including transport of bile salts, nucleosides, chloride ions, cholesterol, toxins, and more. Predominant among this group are MDR1 (which encodes the P-glycoprotein, NP_000918), the MDR-related proteins (MRP1-7), and the breast cancer resistance protein (BCRP). In general, these transporters share a common structure, with each protein containing a pair of ATP-binding domains (also known as nucleotide binding folds, NBF) and two sets of transmembrane (TM) domains, each of which typically contains six membrane-spanning α-helices. The genes encoding this class of transporter are organized as either full transporters (*i.e.* containing two TM and two NBF domains) or as half transporters that assemble as either homodimers or heterodimers to create functional transporters. As a whole, members of the family are widely dispersed throughout the genome and show a high degree of amino acid sequence identify among eukaryotes.

ABC-transporters have been implicated in several human diseases. For instance, molecular efflux pumps of this type play a major role in the development of drug resistance exhibited by a variety of cancers and pathogenic microorganisms. In the case of human cancers, increased expression of the MDR1 gene and related pumps have been observed to generate drug resistance to a broad collection of commonly used chemotherapeutics including doxorubicin, daunorubicin, vinblastine, vincristine, colchicines. In addition to the contribution these transporters make to the development of multi-drug resistance, there are currently 13 human genetic diseases associated with defects in 14 different transporters. The most common of these conditions include cystic fibrosis, Stargardt disease, age-related macular degeneration, adrenoleukodystrophy, Tangier disease, Dubin-Johnson syndrome and progressive familial intrahepatic cholestasis. For this reason, siRNAs directed against members of this, and related, families are potentially valuable research and therapeutic tools.

With respect to channels, analysis of Drosophila mutants has enabled the initial molecular isolation and characterization of several distinct channels including (but not limited to) potassium (K+) channels. This list includes shaker (Sh), which encodes a voltage activated K^+ channel, slowpoke (Slo), a Ca^{2+} activated K^+ channel, and ether-a-go-go (Eag). The Eag family is further divided into three subfamilies: Eag, Elk (eag-like K channels), and Erg (Eag related genes).

The Erg subfamily contains three separate family members (Erg1-3) that are distantly related to the sh family of voltage activated K^+ channels. Like sh, erg polypetides contain the classic six membrane spanning architecture of K^+ channels (S1-S6) but differ in that each includes a segment associated with the C-terminal cytoplasmic region that is homologous to cyclic nucleotide binding domains (cNBD). Like many isolated ion channel mutants, erg mutants are temperature-sensitive paralytics, a phenotype caused by spontaneous repetitive firing (hyperactivity) in neurons and enhanced transmitter release at the neuromuscular junction.

Initial studies on the tissue distribution of all three members of the erg subfamily show two general patterns of expression. Erg1 and erg3 are broadly expressed throughout the nervous system and are observed in the heart, the superior mesenteric ganglia, the celiac ganglia, the retina, and the brain. In contrast, erg2 shows a much more restricted pattern of expression and is only observed in celiac ganglia and superior mesenteric ganglia. Similarly, the kinetic properties of the three erg potassium channels are not homogeneous. Erg1 and erg2 channels are relatively slow activating delayed rectifiers whereas the erg3 current activates rapidly and then exhibits a predominantly transient component that decays to a sustained plateau. The current properties of all three channels are sensitive to methanesulfonanilides, suggesting a high degree of conservation in the pore structure of all three proteins.

Recently, the erg family of K^+ channels has been implicated in human disease. Consistent with the observation that erg1 is expressed in the heart, single strand conformation polymorphism and DNA sequence analyses have identified HERG (human erg1) mutations in six long-QT-syndrome (LQT) families, an inherited disorder that results in sudden death from a ventricular tachyarrythmia. Thus siRNA

directed against this group of molecules (*e.g.* KCNH1-8) will be of extreme therapeutic value.

Another group of channels that are potential targets of siRNAs are the CLCA family that mediate a Ca^{2+}-activated Cl^- conductance in a variety of tissues. To date, two bovine (bCLC1; bCLCA2 (Lu-ECAM-1)), three mouse (mCLCA1; mCLCA2; mCLCA3) and four human (hCLCA1; hCLCA2; hCLCA3; hCLCA4) CLCA family members have been isolated and patch-clamp studies with transfected human embryonic kidney (HEK-293) cells have shown that bCLCA1, mCLCA1, and hCLCA1 mediate a Ca^{2+}-activated Cl^- conductance that can be inhibited by the anion channel blocker DIDS and the reducing agent dithiothreitol (DTT).

The protein size, structure, and processing seem to be similar among different CLCA family members and has been studied in greatest detail for Lu-ECAM-1. The Lu-ECAM-1 open reading frame encodes a precursor glycoprotein of 130 kDa that is processed to a 90-kDa amino-terminal cleavage product and a group of 30- to 40-kDa glycoproteins that are glycosylation variants of a single polypeptide derived from its carboxy terminus. Both subunits are associated with the outer cell surface, but only the 90-kDa subunit is thought to be anchored to the cell membrane via four transmembrane domains.

Although the protein processing and function appear to be conserved among CLCA homologs, significant differences exist in their tissue expression patterns. For example, bovine Lu-ECAM-1 is expressed primarily in vascular endothelia, bCLCA1 is exclusively detected in the trachea, and hCLCA1 is selectively expressed in a subset of human intestinal epithelial cells. Thus the emerging picture is that of a multigene family with members that are highly tissue specific, similar to the ClC family of voltage-gated Cl^- channels. The human channel, hCLCA2, is particular interesting from a medical and pharmacological standpoint. CLCA2 is expressed on the luminal surface of lung vascular endothelia and serves as an adhesion molecule for lung metastatic cancer cells, thus mediating vascular arrest and lung colonization. Expression of this molecule in normal mammary epithelium is consistently lost in human breast cancer and in nearly all tumorigenic breast cancer cell lines. Moreover,

re-expression of hCLCA2 in human breast cancer cells abrogates tumorigenicity in nude mice, implying that hCLCA2 acts as a tumour suppressor in breast cancer. For these reasons, siRNA directed against CLCA family members and related channels may prove to be valuable in research and therapeutic venues.

Transporters Involved in Synaptic Transmission.

Synaptic transmission involves the release of a neurotransmitter into the synaptic cleft, interaction of that transmitter with a postsynaptic receptor, and subsequent removal of the transmitter from the cleft. In most synapses the signal is terminated by a rapid reaccumulation of the neurotransmitter into presynaptic terminals. This process is catalyzed by specific neurotransmitter transporters that are often energized by the electrochemical gradient of sodium across the plasma membrane of the presynaptic cells.

Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. The inhibitory action of GABA, mediated through $GABA_A$/ $GABA_B$ receptors, and is regulated by GABA transporters (GATs), integral membrane proteins located perisynaptically on neurons and glia. So far four different carriers (GAT1-GAT4) have been cloned and their cellular distribution has been partly worked out. Comparative sequence analysis has revealed that GABA transporters are related to several other proteins involved in neurotransmitter uptake including gamma-aminobutyric acid transporters, monoamine transporters, amino acid transporters, certain "orphan" transporters, and the recently discovered bacterial transporters. Each of these proteins has a similar 12 transmembrane helices topology and relies upon the Na+/Cl- gradient for transport function. Transport rates are dependent on substrate concentrations, with half-maximal effective concentrations for transport frequently occurring in the submicromolar to low micromolar range. In addition, transporter function is bidirectional, and non-vesicular efflux of transmitter may contribute to ambient extracellular transmitter levels.

Recent evidence suggests that GABA transporters, and neurotransmitter transporters in general, are not passive players in regulating neuronal signaling; rather, transporter function can be altered by a variety of initiating factors and signal transduction cascades. In general, this functional regulation occurs in two ways,

either by changing the rate of transmitter flux through the transporter or by changing the number of functional transporters on the plasma membrane. A recurring theme in transporter regulation is the rapid redistribution of the transporter protein between intracellular locations and the cell surface. In general, this functional modulation occurs in part through activation of second messengers such as kinases, phosphatases, arachidonic acid, and pH. However, the mechanisms underlying transporter phosphorylation and transporter redistribution have yet to be fully elucidated.

GABA transporters play a pathophysiological role in a number of human diseases including temporal lobe epilepsy and are the targets of pharmacological interventions. Studies in seizure sensitive animals show some (but not all) of the GAT transporters have altered levels of expression at times prior to and post seizure, suggesting this class of transporter may affect epileptogenesis, and that alterations following seizure may be compensatory responses to modulate seizure activity. For these reasons, siRNAs directed against members of this family of genes (including but not limited to SLCG6A1-12) may prove to be valuable research and therapeutic tools.

Organic Ion Transporters.

The human body is continuously exposed to a great variety of xenobiotics, via food, drugs, occupation, and environment. Excretory orgāns such as kidney, liver, and intestine defend the body against the potentially harmful effects of these compounds by transforming them into less active metabolites that are subsequently secreted from the system.

Carrier-mediated transport of xenobiotics and their metabolites exist for the active secretion of organic anions and cations. Both systems are characterized by a high clearance capacity and tremendous diversity of substances accepted, properties that result from the existance of multiple transporters with overlapping substrate specificities. The class of organic anion transporters plays a critical role in the elimination of a large number of drugs (*e.g.*, antibiotics, chemotherapeutics, diuretics, nonsteroidal anti-inflammatory drugs, radiocontrast agents, cytostatics); drug metabolites (especially conjugation products with glutathione, glucuronide, glycine, sulfate, acetate); and toxicants and their metabolites (*e.g.*, mycotoxins, herbicides,

plasticizers, glutathione *S*-conjugates of polyhaloalkanes, polyhaloalkenes, hydroquinones, aminophenols), many of which are specifically harmful to the kidney.

Over the past couple of years the number of identified anion transporting molecules has grown tremendously. Uptake of organic anions (OA^-) across the basolateral membrane is mediated by the classic sodium-dependent organic anion transport system, which includes α-ketoglutarate (α-KG^{2-})/OA^- exchange via the organic anion transporter (OAT1) and sodium-ketoglutarate cotransport via the Na^+/dicarboxylate cotransporter (SDCT2). The organic anion transporting polypeptide, Oatp1, and the kidney-specific OAT-K1 and OAT-K2 are seen as potential molecules that mediate facilitated OA^- efflux but could also be involved in reabsorption via an exchange mechanism. Lastly the PEPT1 and PEPT2 mediate luminal uptake of peptide drugs, whereas CNT1 and CNT2 are involved in reabsorption of nucleosides

The organic anion-transporting polypeptide 1 (Oatp1) is a Na^+- and ATP-independent transporter originally cloned from rat liver. The tissue distribution and transport properties of the Oatp1 gene product are complex. Oatp1 is localized to the basolateral membrane of hepatocytes, and is found on the apical membrane of S3 proximal tubules. Studies with transiently transfected cells (*e.g.* HeLa cells) have indicated that Oatp1 mediates transport of a variety of molecules including taurocholate, estrone-3-sulfate, aldosterone, cortisol, and others. The observed uptake of taurocholate by Oatp1 expressed in *X. laevis* oocytes is accompanied by efflux of GSH, suggesting that transport by this molecule may be glutathione dependent.

Computer modeling suggests that members of the Oatp family are highly conserved, hydrophobic, and have 12 transmembrane domains. Decreases in expression of Oatp family members have been associated with cholestatic liver diseases and human hepatoblastomas, making this family of proteins of key interest to researchers and the medical community. For these reasons, siRNAs directed against OAT family members (including but not limited to SLC21A2, 3, 6, 8, 9, 11, 12, 14, 15, and related transporters) are potentially useful as research and therapeutic tools.

Nucleoside transporters.

Nucleoside transporters play key roles in physiology and pharmacology. Uptake of exogenous nucleosides is a critical first step of nucleotide synthesis in tissues such as bone marrow and intestinal epithelium and certain parasitic organisms that lack *de novo* pathways for purine biosynthesis. Nucleoside transporters also control the extracellular concentration of adenosine in the vicinity of its cell surface receptors and regulate processes such as neurotransmission and cardiovascular activity. Adenosine itself is used clinically to treat cardiac arrhythmias, and nucleoside transport inhibitors such as dipyridamole, dilazep, and draflazine function as coronary vasodilators.

In mammals, plasma membrane transport of nucleosides is brought about by members of the concentrative, Na^+-dependent (CNT) and equilibrative, Na^+-independent (ENT) nucleoside transporter families. CNTs are expressed in a tissue-specific fashion; ENTs are present in most, possibly all, cell types and are responsible for the movement of hydrophilic nucleosides and nucleoside analogs down their concentration gradients. In addition, structure/function studies of ENT family members have predicted these molecules to contain eleven transmembrane helical segments with an amino terminus that is intracellular and a carboxyl terminus that is extracellular. The proteins have a large glycosylated loop between TMs 1 and 2 and a large cytoplasmic loop between TMs 6 and 7. Recent investigations have implicated the TM 3-6 region as playing a central role in solute recognition. The medical importance of the ENT family of proteins is broad. In humans adenosine exerts a range of cardioprotective effects and inhibitors of ENTs are seen as being valuable in alleviating a variety of cardio/cardiovascular ailments. In addition, responses to nucleoside analog drugs has been observed to vary considerably amongst *e.g.* cancer patients. While some forms of drug resistance have been shown to be tied to the up-regulation of ABC-transporters (*e.g.* MDR1), resistance may also be the result of reduced drug uptake (*i.e.* reduced ENT expression). Thus, a clearer understanding of ENT transporters may aid in optimizing drug treatments for patients suffering a wide range of malignancies. For these reasons, siRNAs directed against this class of molecules (including SLC28A1-3, SLC29A1-4, and related molecules) may be useful as therapeutic and research tools.

Sulfate Transporters.

All cells require inorganic sulfate for normal function. Sulfate is the fourth most abundant anion in human plasma and is the major source of sulfur in many organisms. Sulfation of extracellular matrix proteins is critical for maintaining normal cartilage metabolism and sulfate is an important constituent of myelin membranes found in the brain

Because sulfate is a hydrophilic anion that cannot passively cross the lipid bilayer of cell membranes, all cells require a mechanism for sulfate influx and efflux to ensure an optimal supply. To date, a variety of sulfate transporters have been identified in tissues from many origins. These include the renal sulfate transporters (NaSi-1 and Sat-1), the ubiquitously expressed diastrophic dysplasia sulfate transporter (DTDST), the intestinal sulfate transporter (DRA), and the erythrocyte anion exchanger (AE1). Most, if not all, of these molecules contain the classic 12 transmembrane spanning domain architecture commonly found amongst members of the anion transporter superfamily.

Recently three different sulfate transporters have been associated with specific human genetic diseases. Family members SLC26A2, SLC26A3, and SLC26A4 have been recognized as the disease genes mutated in diastrophic dysplasia, congenital chloride diarrhea (CLD), and Pendred syndrome (PDS), respectively. DTDST is a particularly complex disorder. The gene encoding this molecule maps to chromosome 5q, and encodes two distinct transcripts due to alternative exon usage. In contrast to other sulfate transporters (*e.g.* Sat-1) anion movement by the DTDST protein is markedly inhibited by either extracellular chloride or bicarbonate. Impaired function of the DTDST gene product leads to undersulfation of proteoglycans and a complex family of recessively inherited osteochondrodysplasias (achondrogenesis type 1B, atelosteogenesis type II, and diastrophic dysplasia) with clinical features including but not limited to, dwarfism, spinal deformation, and specific joint abnormalities. Interestingly, while epidemiological studies have shown that the disease occurs in most populations, it is particularly prevalent in Finland owing to an apparent founder effect. For these reasons, siRNAs directed against this class of genes (including but not limited to SLC26A1-9, and related molecules) may be potentially helpful in both therapeutic and research venues.

Ion Exchangers

Intracellular pH regulatory mechanisms are critical for the maintenance of countless cellular processes. For instance, in muscle cells, contractile processes and metabolic reactions are influenced by pH. During periods of increased energy demands and ischemia, muscle cells produce large amounts of lactic acid that, without quick and efficient disposal, would lead to acidification of the sarcoplasm.

Several different transport mechanisms have evolved to maintain a relatively constant intracellular pH. The relative contribution of each of these processes varies with cell type, the metabolic requirements of the cell, and the local environmental conditions. Intracellular pH regulatory processes that have been characterized functionally include but are not limited to the Na^+/H^+ exchange, the $Na(HCO_3)_n$ cotransport, and the Na^+-dependent and -independent Cl^-/base exchangers. As bicarbonate and CO_2 comprise the major pH buffer of biological fluids, sodium biocarbonate cotransporters (NBCs) are critical. Studies have shown that these molecules exist in numerous tissues including the kidney, brain, liver, cornea, heart, and lung, suggesting that NBCs play an important role in mediating HCO_3^- transport in both epithelial as well as nonepithelial cells.

Recent molecular cloning experiments have identified the existence of four NBC isoforms (NBC1, 2, 3 and 4) and two NBC-related proteins, AE4 and NCBE (Anion Exchanger 4 and Na-dependent Chloride-Bicarbonate Exchanger). The secondary structure analyses and hydropathy profile of this family predict them to be intrinsic membrane proteins with 12 putative transmembrane domains and several family members exhibit *N*-linked glycosylation sites, protein kinases A and C, casein kinase II, and ATP/GTP-binding consensus phosphorylation sites, as well as potential sites for myristylation and amidation. AE4 is a relatively recent addition to this family of proteins and shows between 30-48% homology with the other family members. When expressed in COS-7 cells and *Xenopus* oocytes AE4 exhibits sodium-independent and DIDS-insensitive anion exchanger activity. Exchangers have been shown to be responsible for a variety of human diseases. For instance, mutations in three genes of the anion transporter family (SLC) are believed to cause known hereditary diseases, including chondrodysplasia (SLC26A2, DTD), diarrhea (A3, down-regulated in adenoma/chloride-losing diarrhea protein: DRA/CLD), and

goiter/deafness syndrome (A4, pendrin). Moreover, mutations in Na+/HCO3 co-transporters have also been associated with various human maladies. For these reasons, siRNAs directed against these sorts of genes (*e.g.* SLC4A4-10, and related genes) may be useful for therapeutic and research purposes.

Receptors Involved in Synaptic Transmission

In all vertebrates, fast inhibitory synaptic transmission is the result of the interaction between the neurotransmitters glycine (Gly) and γ-aminobutyric acid (GABA) and their respective receptors. The strychnine-sensitive glycine receptor is especially important in that it acts in the mammalian spinal cord and brain stem and has a well-established role in the regulation of locomotor behavior.

Glycine receptors display significant sequence homology to several other receptors including the nicotinic acetylcholine receptor, the aminobutyric acid receptor type A ($GABA_AR$), and the serotonin receptor type 3 ($5\text{-}HT_3R$) subunits. As members of the superfamily of ligand-gated ion channels, these polypeptides share common topological features. The glycine receptor is composed of two types of glycosylated integral membrane proteins (α1-α4 and β) arranged in a pentameric suprastructure. The alpha subunit encodes a large extracellular, N-terminal domain that carries the structural determinants essential for agonist and antagonist-binding, followed by four transmembrane spanning regions (TM1-TM4), with TM2 playing the critical role of forming the inner wall of the chloride channel.

The density, location, and subunit composition of glycine neurotransmitter receptors changes over the course of development. It has been observed that the amount of GlyR gene translation (assessed by the injection of developing rat cerebral cortex mRNA into *Xenopus* oocytes) decreases with age, whereas that of GABARs increases. In addition, the type and location of mRNAs coding for GlyR changes over the course of development. For instance in a study of the expression of alpha 1 and alpha 2 subunits in the rat, it was observed that (in embryonic periods E11-18) the mantle zone was scarce in the alpha 1 mRNA, but the germinal zone (matrix layer) at E11-14 expressed higher levels of the message. At postnatal day 0 (P0), the alpha 1 signals became manifested throughout the gray matter of the spinal cord. By contrast,

the spinal tissues at P0 exhibited the highest levels of alpha 2 mRNA, which decreased with the postnatal development.

In both, man and mouse mutant lines, mutations of GlyR subunit genes result in hereditary motor disorders characterized by exaggerated startle responses and increased muscle tone. Pathological alleles of the *Glra1* gene are associated with the murine phenotypes *oscillator (spd^{ot})* and *spasmodic (spd)*. Similarly, a mutant allele of *Glrb* has been found to underly the molecular pathology of the *spastic* mouse *(spa)*. Resembling the situation in the mouse, a variety of *GLRA1* mutant alleles have been shown to be associated with the human neurological disorder hyperekplexia or startle disease. For these reasons, siRNA directed against glycine receptors (GLRA1-3, GLRB, and related molecules), glutamate receptors, GABA receptors, ATP receptors, and related neurotransmitter receptor molecules may be valuable therapeutic and research reagents.

Proteases

Kallikreins

One important class of proteases are the kallikreins, serine endopeptidases that split peptide substrates preferentially on the C-terminal side of internal arginyl and lysyl residues. Kallikreins are generally divided into two distinct groups, plasma kallikreins and tissue kallikreins. Tissue kallikreins represent a large group of enzymes that have substantial similarities at both the gene and protein level. The genes encoding this group are frequently found on a single chromosome, are organized in clusters, and are expressed in a broad range of tissues (*e.g.* pancreas, ovaries, breast). In contrast, the plasma form of the enzyme is encoded by a single gene (*e.g.* KLK3) that has been localized to chromosome 4q34-35 in humans. The gene encoding plasma kallikrein is expressed solely in the liver, contains 15 exons, and encodes a glycoprotein that is translated as a preprotein called prekallikrein.

Kallikreins are believed to play an important role in a host of physiological events For instance, the immediate consequence of plasma prekallikrein activation is the cleavage of high molecular weight kininogen (HK) and the subsequent liberation of bradykinin, a nine amino acid vasoactive peptide that is an important mediator of

inflammatory responses. Similarly, plasma kallikrein promotes single-chain urokinase activation and subsequent plasminogen activation, events that are critical to blood coaggulation and wound healing.

Disruptions in the function of kallikreins have been implicated in a variety of pathological processes including imbalances in renal function and inflammatory processes. For these reasons, siRNAs directed against this class of genes (*e.g.* KLK1-15) may prove valuable in both research and therapeutic settings.

ADAM Proteins

The process of fertilization takes place in a series of discrete steps whereby the sperm interacts with; i) the cumulus cells and the hyaluronic acid extracellular matrix (ECM) in which they are embedded, ii) the egg's own ECM, called the *zona pellucida* (ZP), and iii) the egg plasma membrane. During the course of these interactions, the "acrosome reaction," the exocytosis of the acrosome vesicle on the head of the sperm, is induced, allowing the sperm to penetrate the ZP and gain access to the perivitelline space. This process exposes new portions of the sperm membrane, including the inner acrosomal membrane and the equatorial segment, regions of the sperm head that can participate in initial gamete membrane binding.

The interactions of the gamete plasma membranes appear to involve multiple ligands and receptors and are frequently compared to leukocyte-endothelial interactions. These interactions lead to a series of signal transduction events in the egg, known as collectively as egg activation and include the initiation of oscillations in intracellular calcium concentration, the exit from meiosis, the entry into the first embryonic mitosis, and the formation of a block to polyspermy via the release of ZP-modifying enzymes from the egg's cortical granules. Ultimately, sperm and egg not only adhere to each other but also go on to undergo membrane fusion, making one cell (the zygote) from two.

Studies on the process of sperm-egg interactions have identified a number of proteins that are crucial for fertilization. One class of proteins, called the ADAM family (A Disintegrin And Metalloprotease), has been found to be important in spermatogenesis and fertilization, as well as various developmental systems including

myogenesis and neurogenesis. Members of the family contain a disintegrin and metalloprotease domain (and therefore have (potentially) both cell adhesion and protease activities), as well as cysteine-rich regions, epidermal growth factor (EGF)-like domains, a transmembrane region, and a cytoplasmic tail. Currently, the ADAM gene family has 29 members and constituents are widely distributed in many tissues including the brain, testis, epididymis, ovary, breast, placenta, liver, heart, lung, bone, and muscle.

One of the best-studied members of the ADAM family is fertilin, a heterodimeric protein comprised of at least two subunits, fertilin alpha and fertilin beta. The fertilin beta gene (ADAM2) has been disrupted with a targeting gene construct corresponding to the exon encoding the fertilin beta disintegrin domain. Sperm from males homozygous for disruptions in this region exhibit defects in multiple facets of sperm function including reduced levels of sperm transit from the uterus to the oviduct, reduced sperm-ZP binding, and reduced sperm-egg binding, all of which contribute to male infertility.

Recently, four new ADAM family members (ADAM 24-27) have been isolated. The deduced amino acid sequences show that all four contain the complete domain organization common to ADAM family members and Northern Blot analysis has shown all four to be specific to the testes. SiRNAs directed against this class of genes (*e.g.* ADAM2 and related proteins) may be useful as research tools and therapeutics directed toward fertility and birth control.

Aminopeptidases

Aminopeptidases are proteases that play critical roles in processes such as protein maturation, protein digestion in its terminal stage, regulation of hormone levels, selective or homeostatic protein turnover, and plasmid stabilization. These enzymes generally have broad substrate specificity, occur in several forms and play a major role in physiological homeostasis. For instance, the effects of bradykinin, angiotensin converting enzyme (ACE), and other vasoactive molecules are muted by one of several peptidases that cleave the molecule at an internal position and eliminate its ability to bind its cognate receptor (*e.g.* for bradykinin, the B2-receptor).

Among the enzymes that can cleave bradykinin is the membrane bound aminopeptidase P, also referred to as aminoacylproline aminopeptidase, proline aminopeptidase; X-Pro aminopeptidase (eukaryote) and XPNPEP2. Aminopeptidase P is an aminoacylproline aminopeptidase specific for NH_2-terminal Xaa-proline bonds. The enzyme i) is a mono-zinc-containing molecule that lacks any of the typical metal binding motifs found in other zinc metalloproteases, ii) has an active-site configuration similar to that of other members of the MG peptidase family, and iii) is present in a variety of tissues including but not limited to the lung, kidney, brain, and intestine.

Aminopeptidases play an important role in a diverse set of human diseases. Low plasma concentrations of aminopeptidase P are a potential predisposing factor for development of angio-oedema in patients treated with ACE inhibitors, and inhibitors of aminopeptidase P may act as cardioprotectors against other forms of illness including, but not limited to myocardial infarction. For these reasons, siRNAs directed against this family of proteins (including but not limited to XPNPEP1 and related proteins) may be useful as research and therapeutic tools.

Serine Proteases

One important class of proteases are the serine proteases. Serine proteases share a common catalytic triad of three amino acids in their active site (serine (nucleophile), aspartate (electrophile), and histidine (base)) and can hydrolyze either esters or peptide bonds utilizing mechanisms of covalent catalysis and preferential binding of the transition state. Based on the position of their introns serine proteases have been classified into a minimum of four groups including those in which 1) the gene has no introns interrupting the exon coding for the catalytic triad (*e.g.* the haptoglobin gene,); 2) each gene contains an intron just downstream from the codon for the histidine residue at the active site, a second intron downstream from the exon containing the aspartic acid residue of the active site and a third intron just upstream from the exon containing the serine of the active site (*e.g.* trypsinogen, chymotrypsinogen, kallikrein and proelastase); 3) the genes contain seven introns interrupting the exons coding the catalytic region (*e.g.* complement factor B gene); and 4) the genes contain two introns resulting in a large exon that contains both the

active site aspartatic acid and serine residues (*e.g.* factor X, factor IX and protein C genes).

Cytotoxic lymphocytes (*e.g.* CD8(+) cytotoxic T cells and natural killer cells) form the major defense of higher organisms against virus-infected and transformed cells. A key function of these cells is to detect and eliminate potentially harmful cells by inducing them to undergo apoptosis. This is achieved through two principal pathways, both of which require direct but transient contact between the killer cell and its target. The first pathway involves ligation of TNF receptor-like molecules such as Fas/CD95 to their cognate ligands, and results in mobilization of conventional, programmed cell-death pathways centered on activation of pro-apoptotic caspases. The second mechanism consists of a pathway whereby the toxic contents of a specialized class of secretory vesicles are introduced into the target cell. Studies over the last two decades have identified the toxic components as Granzymes, a family of serine proteases that are expressed exclusively by cytotoxic T lymphocytes and natural killer (NK) cells. These agents are stored in specialized lytic granules and enter the target cell via endocytosis. Like caspases, cysteine proteases that play an important role in apoptosis, granzymes can cleave proteins after acidic residues, especially aspartic acid, and induce apoptosis in the recipient cell.

Granzymes have been grouped into three subfamilies according to substrate specificity. Members of the granzyme family that have enzymatic activity similar to the serine protease chymotrypsin are encoded by a gene cluster termed the 'chymase locus'. Similarly, granzymes with trypsin-like specificities are encoded by the 'tryptase locus', and a third subfamily cleaves after unbranched hydrophobic residues, especially methionine, and are encoded by the 'Met-ase locus'. All granzymes are synthesized as zymogens and, after clipping of the leader peptide, obtain maximal enzymatic activity subsequent to the removal of an amino-terminal dipeptide.

Granzymes have been found to be important in a number of important biological functions including defense against intracellular pathogens, graft versus host reactions, the susceptibility to transplantable and spontaneous malignancies, lymphoid homeostasis, and the tendency toward auto-immune diseases. For these

reasons, siRNAs directed against granszymes (*e.g.* GZMA, GZMB, GZMH, GZHK, GZMM) and related serine proteases may be useful research and therapeutic reagents.

Kinases

Protein Kinases (PKs) have been implicated in a number of biological processes. Kinase molecules play a central role in modulating cellular physiology and developmental decisions, and have been implicated in a large list of human maladies including cancer, diabetes, and others.

During the course of the last three decades, over a hundred distinct protein kinases have been identified, all with presumed specific cellular functions. A few of these enzymes have been isolated to sufficient purity to perform *in vitro* studies, but most remain intractable due to the low abundance of these molecules in the cell. To counter this technical difficulty, a number of protein kinases have been isolated by molecular cloning strategies that utilize the conserved sequences of the catalytic domain to isolate closely related homologs. Alternatively, some kinases have been purified (and subsequently studied) based on their interactions with other molecules.

p58 is a member of the p34cdc2-related supergene family and contains a large domain that is highly homologous to the cell division control kinase, cdc2. This new cell division control-related protein kinase was originally identified as a component of semipurified galactosyltransferase; thus, it has been denoted galactosyltransferase-associated protein kinase (GTA-kinase). GTA-kinase has been found to be expressed in both adult and embryonic tissues and is known to phosphorylate a number of substrates, including histone H1, and casein. Interestingly enough, over expression of this molecule in CHO cells has shown that elevated levels of p58 result in a prolonged late telophase and an early G1 phase, thus hinting of an important role for GTA-kinase in cell cycle regulation.

Cyclin Dependent Kinases

The cyclin-dependent kinases (Cdks) are a family of highly conserved serine/threonine kinases that mediate many of the cell cycle transitions that occur during duplication. Each of these Cdk catalytic subunits associates with a specific

subset of regulatory subunits, termed cyclins, to produce a distinct Cdk·cyclin kinase complex that, in general, functions to execute a unique cell cycle event.

Activation of the Cdk·cyclin kinases during cellular transitions is controlled by a variety of regulatory mechanisms. For the Cdc2·cyclin B complex, inhibition of kinase activity during S phase and G_2 is accomplished by phosphorylation of two Cdc2 residues, Thr^{14} and Tyr^{15}, which are positioned within the ATP-binding cleft. Phosphorylation of Thr^{14} and/or Tyr^{15} suppresses the catalytic activity of the molecule by disrupting the orientation of the ATP present within this cleft. In contrast, the abrupt dephosphorylation of these residues by the Cdc25 phosphatase results in the rapid activation of Cdc2·cyclin B kinase activity and subsequent downstream mitotic events. While the exact details of this pathway have yet to be elucidated, it has been proposed that Thr^{14}/Tyr^{15} phosphorylation functions to permit a cell to attain a critical concentration of inactive Cdk·cyclin complexes, which, upon activation, induces a rapid and complete cell cycle transition. Furthermore, there is evidence in mammalian cells that Thr^{14}/Tyr^{15} phosphorylation also functions to delay Cdk activation after DNA damage.

The *Schizosaccharomyces pombe wee1* gene product was the first kinase identified that is capable of phosphorylating Tyr^{15} in Cdc2. Homologs of the Wee1 kinase have been subsequently identified and biochemically characterized from a wide range of species including human, mouse, frog, *Saccharomyces cerevisiae*, and *Drosophila*. In vertebrate systems, where Thr^{14} in Cdc2 is also phosphorylated, the Wee1 kinase was capable of phosphorylating Cdc2 on Tyr^{15}, but not Thr^{14}, indicating that another kinase was responsible for Thr^{14} phosphorylation. This gene, Myt1 kinase, was recently isolated from the membrane fractions of *Xenopus* egg extracts and has been shown to be capable of phosphorylating Thr^{14} and, to a lessor extent, Tyr^{15} in Cdc2. A human Myt1 homolog displaying similar properties has been isolated, as well as a non-membrane-associated molecule with Thr^{14} kinase activity.

In the past decade it has been shown that cancer can originate from overexpression of positive regulators, such as cyclins, or from underexpression of negative regulators (*e.g.* p16 (INK4a), p15 (INK4b), p21 (Cip1)). Inhibitors such as Myt1 are the focus of much cancer research because they are capable of controlling

cell cycle proliferation, now considered the Holy Grail for cancer treatment. For these reasons, siRNA directed against kinases and kinase inhibitors including but not limited to ABL1, ABL2, ACK1, ALK, AXL, BLK, BMX, BTK, C20orf64, CSF1R, SCK, DDR1, DDR2, DKFZp761P1010, EGFR, EPHA1, EPHA2, EPHA3, EPHA4, EPHA7, EPHA8, EPHB1, EPHB2, EPHB3, EPHB4. EPHB6, ERBB2, ERBB3, ERBB4, FER, FES, FGFR1, FGFR2, FGFR3, FGFR4, FGR, FLT1, FLT3, FLT4, FRK, FYN, HCK, IGF1R, INSR, ITK, JAK1, JAK2, JAK3, KDR, KIAA1079, KIT, LCK, LTK, LYN, MATK, MERTK, MET, MST1R, MUSK, NTRK1, NTRK2, NTRK3, PDGFRA, PDGFRB, PTK2, PTK2B, PTK6, PTK7, PTK9, PTK9L, RET, ROR1, ROR2, ROS1, RYK, SRC, SYK, TEC, TEK, TIE, TNK1, TXK, TYK2, TYRO3, YES1, and related proteins, may be useful for research and therapeutic purposes.

G Protein Coupled Receptors

One important class of genes to which siRNAs can be directed are G-protein coupled receptors (GPCRs). GPCRs constitute a superfamily of seven transmembrane spanning proteins that respond to a diverse array of sensory and chemical stimuli, such as light, odor, taste, pheromones, hormones and neurotransmitters. GPCRs play a central role in cell proliferation, differentiation, and have been implicated in the etiology of disease.

The mechanism by which G protein-coupled receptors translate extracellular signals into cellular changes was initially envisioned as a simple linear model: activation of the receptor by agonist binding leads to dissociation of the heterotrimeric GTP-binding G protein (Gs, Gi, or Gq) into its alpha and beta/gamma subunits, both of which can activate or inhibit various downstream effector molecules. More specifically, activation of the GPCR induces a conformational change in the Gα subunit, causing GDP to be released and GTP to be bound in its place. The Gα and Gβγ subunits then dissociate from the receptor and interact with a variety of effector molecules. For instance in the case of the Gs family, the primary function is to stimulate the intracellular messenger adenylate cyclase (AC), which catalyzes the conversion of cytoplasmic ATP into the secondary messenger cyclic AMP (cAMP). In contrast, the Gi family inhibits this pathway and the Gq family activates

phospholipases C (PLC), which cleaves phosphatidylinositol 4,5, bisphosphate (PIP2) to generate inositol-1,4,5-phosphate (IP3) and diacylglycerol (DAG).

More recently, studies have shown that the functions of GPCRs are not limited to their actions on G-proteins and that considerable cross-talk exists between this diverse group of receptor molecules and a second class of membrane bound proteins, the receptor tyrosine kinases (RTKs). A number of GPCRs such as endothelin-1, thrombin, bombesin, and dopamine receptors can activate MAPKs, a downstream effector of the RTK/Ras pathway. Interestingly, the interaction between these two families is not unidirectional and RTKs can also modulate the activity of signaling pathways traditionally thought to be controlled exclusively by ligands that couple to GPCRs. For instance, EGF, which normally activates the MAPK cascade via the EGF receptor can stimulate adenylate cyclase activity by activating Gαs.

There are dozens of members of the G Protein-Coupled Receptor family that have emerged as prominent drug targets in the last decade. One non-limiting list of potential GPCR-siRNA targets is as follows:

CMKLR1

CML1/ CMKLR1 (Accession No. Q99788) is a member of the chemokine receptor family of GPCRs that may play a role in a number of diseases including those involved in inflammation and immunological responses (*e.g.* asthma, arthritis). For this reason, siRNA directed against this protein may prove to be important therapeutic reagents.

Studies of juvenile-onset neuronal ceroid lipofuscinosis (JNCL, Batten disease), the most common form of childhood encephalopathy that is characterized by progressive neural degeneration, show that it is brought on by mutations in a novel lysosomal membrane protein (CLN3). In addition to being implicated in JNCL, CLN3 (GPCR-like protein, Accession No. A57219) expression studies have shown that the CLN3 mRNA and protein are highly over-expressed in a number of cancers (*e.g.* glioblastomas, neuroblastomas, as well as cancers of the prostate, ovaries, breast, and colon) suggesting a possible contribution of this gene to tumor growth. For this

reason, siRNA directed against this protein may prove to be important therapeutic reagents.

CLACR

The calcitonin receptor (CTR/ CALCR, Accession No. NM_001742) belongs to "family B" of GPCRs which typically recognized regulatory peptides such as parathyroid hormone, secretin, glucagons and vasoactive intestinal polypeptide. Although the CT receptor typically binds to calcitonin (CT), a 32 amino acid peptide hormone produced primarily by the thyroid, association of the receptor with RAMP (Receptor Activity Modulating Protein) enables it to readily bind other members of the calcitonin peptide family including amylin (AMY) and other CT gene-related peptides (*e.g.* αCGRP and βCGRP). While the primary function of the calcitonin receptor pertains to regulating osteoclast mediated bone resorption and enhanced Ca^{+2} excretion by the kidney, recent studies have shown that CT and CTRs may play an important role in a variety of processes as wide ranging as embryonic/foetal development and sperm function/physiology. In addition, studies have shown that patients with particular CTR genotypes may be at higher risk to lose bone mass and that this GPCR may contribute to the formation of calcium oxalate urinary stones. For this reason, siRNA directed against CTR may be useful as therapeutic reagents.

OXTR

The human oxytocin receptor (OTR, OXTR) is a 389 amino acid polypeptide that exhibits the seven transmembrane domain structure and belongs to the Class-I (rhodopsin-type) family of G-protein coupled receptors. OTR is expressed in a wide variety of tissues throughout development and mediates physiological changes through G(q) proteins and phospholipase C-beta. Studies on the functions of oxytocin and the oxytocin receptor have revealed a broad list of duties. OT and OTR play a role in a host of sexual, maternal and social behaviors that include egg-laying, birth, milk-letdown, feeding, grooming, memory and learning. In addition, it has been hypothesized that abnormalities in the functionality of oxytocin-OTR receptor-ligand system can lead to a host of irregularities including compulsive behavior, eating disorders (such as anorexia), depression, and various forms of neurodegenerative

diseases. For these reasons, siRNA directed against this gene (NM_000916) may play an important role in combating OTR-associated illnesses.

EDG GPCRs

Lysophosphatidic acid and other lipid-based hormones/growth factors induce their effects by activating signaling pathways through the G-protein coupled receptors (GPCRs) and have been observed to play important roles in a number of human diseases including cancer, asthma, and vascular pathologies. For instance, during studies of immunoglobulin A nephropathy (IgAN), researchers have observed an enhanced expression of EDG5 (NP_004221) suggesting a contribution of this gene product in the development of IgAN. For that reasons, siRNA directed against Edg5 (NM_004230), Edg4 (NM_004720), Edg7 (Nm_012152) and related genes may play an important role in combating human disease.

Genes Involved in Cholesterol Signaling and Biosynthesis

Studies on model genetic organisms such as *Drosophila* and *C. elegans* have led to the identification of a plethora of genes that are essential for early development. Mutational analysis and ectopic expression studies have allowed many of these genes to be grouped into discreet signal transduction pathways and have shown that these elements play critical roles in pattern formation and cell differentiation. Disruption of one or more of these genes during early stages of development frequently leads to birth defects whereas as alteration of gene function at later stages in life can result in tumorigenesis.

One critical set of interactions known to exist in both invertebrates and vertebrates is the Sonic Hedgehog-Patched-Gli pathway. Originally documented as a *Drosophila* segmentation mutant, several labs have recently identified human and mouse orthologs of many of the pathways members and have successfully related disruptions in these genes to known diseases. Pathway activation is initiated with the secretion of Sonic hedgehog. There are three closely related members of the Shh family (Sonic hedgehog, Desert, and Indian) with Shh being the most widely expressed form of the group. The Shh gene product is secreted as a small pro-signal molecule. To successfully initiate its developmental role, Shh is first cleaved, whereupon the N-terminal truncated fragment is covalently modified with cholesterol.

The addition of the sterol moiety promotes the interaction between Shh and its cognate membrane bound receptor, Patched (Ptch). There are at least two isoforms of the Patched gene, Ptch1 and Ptch2. Both isoforms contain a sterol-sensing domain (SSD); a roughly 180 amino acid cluster that is found in at least seven different classes of molecules including those involved in cholesterol biosynthesis, vesicular traffic, signal transduction, cholesterol transport, and sterol homeostasis. In the absence of Shh, the Patched protein is a negative regulator of the pathway. In contrast, binding of Shh-cholesterol to the Patched receptor releases the negative inhibition which that molecule enforces on a G-protein coupled receptor known as Smoothened. Subsequent activation of Smoothened (directly or indirectly) leads to the triggering of a trio of transcription factors that belong to the Gli family. All three factors are relatively large, contain a characteristic C2-H2 zinc-finger-pentamer, and recognize one of two consensus sequences (SEQ. ID NO. 0463 GACCACCCA or SEQ. ID NO. 0464 GAACCACCCA). In the absence of Shh, Gli proteins are cleaved by the proteosome and the C-terminally truncated fragment translocates to the nucleus and acts as a dominant transcription repressor. In the presence of Shh-cholesterol, Gli repressor formation is inhibited and full-length Gli functions as a transcriptional activator.

Shh and other members of the Shh-PTCH-Gli pathway are expressed in a broad range of tissues (*e.g.* the notochord, the floorplate of the neural tube, the brain, and the gut) at early stages in development. Not surprisingly, mutations that lead to altered protein expression or function have been shown to induce developmental abnormalities. Defects in the human Shh gene have been shown to cause holoprosencephaly, a midline defect that manifests itself as cleft lip or palate, CNS septation, and a wide range of other phenotypes. Interestingly, defects in cholesterol biosynthesis generate similar Shh-like disorders (*e.g.* Smith-Lemli-Opitz syndrome) suggesting that cholesterol modification of the Shh gene product is crucial for pathway function. Both the Patched and Smoothened genes have also been shown to be clinically relevant with Smoothened now being recognized as an oncogene that, like PTCH-1 and PTCH-2, is believed to be the causative agent of several forms of adult tumors. For these reasons, siRNA directed against Smoothened (SMO, NM_005631), Patched (PTCH, nm_000264), and additional genes that participate in

cholesterol signaling, biosynthesis, and degradation, have potentially useful research and therapeutic applications.

Targeted Pathways.

In addition to targeting siRNA against one or more members of a family of proteins, siRNA can be directed against members of a pathway. Thus, for instance, siRNA can be directed against members of a signal transduction pathway (*e.g.* the insulin pathway, including AKT1-3, CBL, CBLB, EIF4EBP1, FOXO1A, FOXO3A, FRAP1, GSK3A, GSK3B, IGF1, IGF1R, INPP5D, INSR, IRS1, MLLT7, PDPK1, PIK3CA, PIK3CB, PIK3R1, PIK3R2, PPP2R2B, PTEN, RPS6, RPS6KA1, RPX6KA3, SGK, TSC1, TSC2, AND XPO1), an apoptotic pathway (CASP3,6,7,8,9, DSH1/2, P110, P85, PDK1/2, CATENIN, HSP90, CDC37, P23, BAD, BCLXL, BCL2, SMAC, and others), pathways, involved in DNA damage, cell cycle, and other physiological (p53,MDM2, CHK1/2, BRCA1/2, ATM, ATR, P15INK4, P27, P21, SKP2, CDC25C/A, 14-3-3, PLK, RB, CDK4, GLUT4, Inos, Mtor, FKBP, PPAR, RXR, ER). Similarly, genes involved in immune system function including TNFR1, IL-IR, IRAK1/2, TRAF2, TRAF6, TRADD, FADD, IKKε, IKKγ, IKKβ, IKKα, IkBα, IkBβ, p50, p65, Rac, RhoA, Cdc42, ROCK, Pak1/2/3/4/5/6, cIAP, HDAC1/2, CBP, β-TrCP, Rip2/4, and others are also important targets for the siRNAs described in this document and may be useful in treating immune system disorders. Genes involved in apoptosis, such as Dsh1/2,PTEN, P110 (pan), P85, PDK1/2, Akt1, Akt2, Akt (pan), $p70^{S6K}$, GSK3β, PP2A (cat), β-catenin, HSP90, Cdc37/p50, P23, Bad, BclxL, Bcl2, Smac/Diablo, and Ask1 are potentially useful in the treatment of diseases that involve defects in programmed cell death (*e.g.* cancer), while siRNA agents directed against p53, MDM2, Chk1/2, BRCA1/2, ATM, ATR, $p15^{INK4}$, P27, P21, Skp2, Cdc25C/A, 14-3-3σ/ε, PLK, Rb, Cdk4, Glut4, iNOS, mTOR, FKBP, PPARγ, RXRα, ERα and related genes may play a critical role in combating diseases associated with disruptions in DNA repair, and cell cycle abnormalities.

Tables VI -Table X below provide examples of useful pools for inhibiting different genes in the human insulin pathway and tyrosine kinase pathways, proteins involved in the cell cycle, the production of nuclear receptors, and other genes. These particular pools are particularly useful in humans, but would be useful in any species

that generates an appropriately homologous mRNA. Further, within each of the listed pools any one sequence maybe used independently but preferably at least two of the listed sequences, more preferably at least three, and most preferably all of the listed sequences for a given gene is present.

Table VI

Gene Name	Acc#	GI	L.L.	Duplex #	Sequence	SEQ. ID NO
AKT1	NM_005163	4885060	207	D-003000-05	GACAAGGACGGGCACATTA	465
AKT1	NM_005163	4885060	207	D-003000-06	GGACAAGGACGGGCACATT	466
AKT1	NM_005163	4885060	207	D-003000-07	GCTACTTCCTCCTCAAGAA	467
AKT1	NM_005163	4885060	207	D-003000-08	GACCGCCTCTCGCTTTGTCA	468
AKT2						
AKT2	NM_001626	6715585	208	D-003001-05	GTACTTCGATGATGAATTT	469
AKT2	NM_001626	6715585	208	D-003001-06	GCAAAGAGGGCATCAGTGA	470
AKT2	NM_001626	6715585	208	D-003001-07	GGGCTAAAGTGACCATGAA	471
AKT2	NM_001626	6715585	208	D-003001-08	GCAGAATGCCAGCTGATGA	472
AKT3						
AKT3	NM_005465	32307164	10000	D-003002-05	GGAGTAAACTGGCAAGATG	473
AKT3	NM_005465	32307164	10000	D-003002-06	GACATTAAATTTCCTCGAA	474
AKT3	NM_005465	32307164	10000	D-003002-07	GACCAAAGCCAAACACATT	475
AKT3	NM_005465	32307164	10000	D-003002-08	GAGGAGAGAATGAATTGTA	476
CBL						
CBL	NM_005188	4885116	867	D-003003-05	GGAGACACATTTCGGATTA	477
CBL	NM_005188	4885116	867	D-003003-06	GATCTGACCTGCAATGATT	478
CBL	NM_005188	4885116	867	D-003003-07	GACAATCCCTCACAATAAA	479
CBL	NM_005188	4885116	867	D-003003-08	CCAGAAAGCTTTGGTCATT	480
CBLB						
CBLB	NM_170662	29366807	868	D-003004-05	GACCATACCTCATAACAAG	481
CBLB	NM_170662	29366807	868	D-003004-06	TGAAAGACCTCCACCAATC	482
CBLB	NM_170662	29366807	868	D-003004-07	GATGAAGGCTCCAGGTGTT	483
CBLB	NM_170662	29366807	868	D-003004-08	TATCAGCATTTACGACTTA	484
EIF4EBP1						
EIF4EBP1	NM_004095	20070179	1978	D-003005-05	GCAATAGCCCAGAAGATAA	485
EIF4EBP1	NM_004095	20070179	1978	D-003005-06	CGCAATAGCCCAGAAGATA	486
EIF4EBP1	NM_004095	20070179	1978	D-003005-07	GAGATGGACATTTAAAGCA	487
EIF4EBP1	NM_004095	20070179	1978	D-003005-08	CAATAGCCCAGAAGATAAG	488
FOXO1A						
FOXO1A	NM_002015	9257221	2308	D-003006-05	CCAGGCATCTCATAACAAA	489
FOXO1A	NM_002015	9257221	2308	D-003006-06	CCAGATGCCTATACAAACA	490
FOXO1A	NM_002015	9257221	2308	D-003006-07	GGAGGTATGAGTCAGTATA	491
FOXO1A	NM_002015	9257221	2308	D-003006-08	GAGGTATGAGTCAGTATAA	492
FOXO3A						
FOXO3A	NM_001455	4503738	2309	D-003007-01	CAATAGCAACAAGTATACC	493
FOXO3A	NM_001455	4503738	2309	D-003007-02	TGAAGTCCAGGACGATGAT	494
FOXO3A	NM_001455	4503738	2309	D-003007-03	TGTCACACTATGGTAACCA	495
FOXO3A	NM_001455	4503738	2309	D-003007-04	TGTTCAATGGGAGCTTGGA	496
FRAP1						
FRAP1	NM_004958	19924298	2475	D-003008-05	GAGAAGAAATGGAAGAAAT	497
FRAP1	NM_004958	19924298	2475	D-003008-06	CCAAAGTGCTGCAGTACTA	498
FRAP1	NM_004958	19924298	2475	D-003008-07	GAGCATGCCGTCAATAATA	499
FRAP1	NM_004958	19924298	2475	D-003008-08	GGTCTGAACTGAATGAAGA	500

GSK3A						
GSK3A	NM_019884	11995473	2931	D-003009-05	GGACAAAGGTGTTCAAATC	501
GSK3A	NM_019884	11995473	2931	D-003009-06	GAACCCAGCTGCCTAACAA	502
GSK3A	NM_019884	11995473	2931	D-003009-07	GCGCACAGCTTCTTTGATG	503
GSK3A	NM_019884	11995473	2931	D-003009-08	GCTCTAGCCTGCTGGAGTA	504
GSK3B						
GSK3B	NM_002093	21361339	2932	D-003010-05	GAAGAAAGATGAGGTCTAT	505
GSK3B	NM_002093	21361339	2932	D-003010-06	GGACCCAAATGTCAAACTA	506
GSK3B	NM_002093	21361339	2932	D-003010-07	GAAATGAACCCAAACTACA	507
GSK3B	NM_002093	21361339	2932	D-003010-08	GATGAGGTCTATCTTAATC	508
IGF1						
IGF1	NM_000618			D-003011-05	GGAAGTACATTTGAAGAAC	509
IGF1	NM_000618			D-003011-06	AGAAGGAAGTACATTTGAA	510
IGF1	NM_000618			D-003011-07	CCTCAAGCCTGCCAAGTCA	511
IGF1	NM_000618			D-003011-08	GGTGGATGCTCTTCAGTTC	512
IGF1R						
IGF1R	NM_000875	11068002	3480	D-003012-05	CAACGAAGCTTCTGTGATG	513
IGF1R	NM_000875	11068002	3480	D-003012-06	GGCCAGAAATGGAGAATAA	514
IGF1R	NM_000875	11068002	3480	D-003012-07	GAAGCACCCTTTAAGAATG	515
IGF1R	NM_000875	11068002	3480	D-003012-08	GCAGACACCTACAACATCA	516
INPP5D						
INPP5D	NM_005541	5031798	3635	D-003013-05	GGAATTGCGTTTACACTTA	517
INPP5D	NM_005541	5031798	3635	D-003013-06	GGAAACTGATCATTAAGAA	518
INPP5D	NM_005541	5031798	3635	D-003013-07	CGACAGGGATGAAGTACAA	519
INPP5D	NM_005541	5031798	3635	D-003013-08	AAACGCAGCTGCCCATCTA	520
INSR						
INSR	NM_000208	4557883	3643	D-003014-05	GGAAGACGTTTGAGGATTA	521
INSR	NM_000208	4557883	3643	D-003014-06	GAACAAGGCTCCCGAGAGT	522
INSR	NM_000208	4557883	3643	D-003014-07	GGAGAGACCTTGGAAATTG	523
INSR	NM_000208	4557883	3643	D-003014-08	GGACGGAACCCACCTATTT	524
IRS1						
IRS1	NM_005544	5031804	3667	D-003015-05	AAAGAGGTCTGGCAAGTGA	525
IRS1	NM_005544	5031804	3667	D-003015-06	GAACCTGATTGGTATCTAC	526
IRS1	NM_005544	5031804	3667	D-003015-07	CCACGGCGATCTAGTGCTT	527
IRS1	NM_005544	5031804	3667	D-003015-08	GTCAGTCTGTCGTCCAGTA	528
MLLT7						
MLLT7	NM_005938	5174578	4303	D-003016-05	GGACTGGACTTCAACTTTG	529
MLLT7	NM_005938	5174578	4303	D-003016-06	CCACGAAGCAGTTCAAATG	530
MLLT7	NM_005938	5174578	4303	D-003016-07	GAGAAGCGACTGACACTTG	531
MLLT7	NM_005938	5174578	4303	D-003016-08	GACCAGAGATCGCTAACCA	532
PDPK1						
PDPK1	NM_002613	4505694	5170	D-003017-05	CAAGAGACCTCGTGGAGAA	533
PDPK1	NM_002613	4505694	5170	D-003017-06	GACCAGAGGCCAAGAATTT	534
PDPK1	NM_002613	4505694	5170	D-003017-07	GGAAACGAGTATCTTATAT	535
PDPK1	NM_002613	4505694	5170	D-003017-08	GAGAAGCGACATATCATAA	536
PIK3CA						
PIK3CA	NM_006218	5453891	5290	D-003018-05	GCTATCATCTGAACAATTA	537
PIK3CA	NM_006218	5453891	5290	D-003018-06	GGATAGAGGCCAAATAATA	538
PIK3CA	NM_006218	5453891	5290	D-003018-07	GGACAACTGTTTCATATAG	539
PIK3CA	NM_006218	5453891	5290	D-003018-08	GCCAGTACCTCATGGATTA	540
PIK3CB						
PIK3CB	NM_006219	5453893	5291	D-003019-05	CGACAAGACTGCCGAGAGA	541
PIK3CB	NM_006219	5453893	5291	D-003019-06	TCAAGTGTCTCCTAATATG	542
PIK3CB	NM_006219	5453893	5291	D-003019-07	GGATTCAGTTGGAGTGATT	543
PIK3CB	NM_006219	5453893	5291	D-003019-08	TTTCAAGTGTCTCCTAATA	544
PIK3R1						

PIK3R1	NM_181504	32455251	5295	D-003020-05	GGAAATATGGCTTCTCTGA	545
PIK3R1	NM_181504	32455251	5295	D-003020-06	GAAAGACGAGAGACCAATA	546
PIK3R1	NM_181504	32455251	5295	D-003020-07	GTAAAGCATTGTGTCATAA	547
PIK3R1	NM_181504	32455251	5295	D-003020-08	GGATCAAGTTGTCAAAGAA	548
PIK3R2						
PIK3R2	NM_005027	4826907	5296	D-003021-05	GGAAAGGCGGGAACAATAA	549
PIK3R2	NM_005027	4826907	5296	D-003021-06	GATGAAGCGTACTGCAATT	550
PIK3R2	NM_005027	4826907	5296	D-003021-07	GGACAGCGAATCTCACTAC	551
PIK3R2	NM_005027	4826907	5296	D-003021-08	GCAAGATCCGAGACCAGTA	552
PPP2R2B						
PPP2R2B	NM_004576	4758953	5521	D-003022-05	GAATGCAGCTTACTTTCTT	553
PPP2R2B	NM_004576	4758953	5521	D-003022-06	GACCGAAGCTGACATTATC	554
PPP2R2B	NM_004576	4758953	5521	D-003022-07	TCGATTACCTGAAGAGTTT	555
PPP2R2B	NM_004576	4758953	5521	D-003022-08	CCTGAAGAGTTTAGAAATA	556
PTEN						
PTEN	NM_000314	4506248	5728	D-003023-05	GTGAAGATCTTGACCAATG	557
PTEN	NM_000314	4506248	5728	D-003023-06	GATCAGCATACACAAATTA	558
PTEN	NM_000314	4506248	5728	D-003023-07	GGCGCTATGTGTATTATTA	559
PTEN	NM_000314	4506248	5728	D-003023-08	GTATAGAGCGTGCAGATAA	560
RPS6						
RPS6	NM_001010	17158043	6194	D-003024-05	GCCAGAAACTCATTGAAGT	561
RPS6	NM_001010	17158043	6194	D-003024-06	GGATATTCCTGGACTGACT	562
RPS6	NM_001010	17158043	6194	D-003024-07	CCAAGGAGAACTGGAGAAA	563
RPS6	NM_001010	17158043	6194	D-003024-08	GCGTATGGCCACAGAAGTT	564
RPS6KA1						
RPS6KA1	NM_002953	20149546	6195	D-003025-05	GATGACACCTTCTACTTTG	565
RPS6KA1	NM_002953	20149546	6195	D-003025-06	GAGAATGGGCTCCTCATGA	566
RPS6KA1	NM_002953	20149546	6195	D-003025-07	CAAGCGGGATCCTTCAGAA	567
RPS6KA1	NM_002953	20149546	6195	D-003025-08	CCACCGGCCTGATGGAAGA	568
RPS6KA3						
RPS6KA3	NM_004586	4759049	6197	D-003026-05	GAAGGGAAGTTGTATCTTA	569
RPS6KA3	NM_004586	4759049	6197	D-003026-06	GAAAGTATGTGTATGTAGT	570
RPS6KA3	NM_004586	4759049	6197	D-003026-07	GGACAGCATCCAAACATTA	571
RPS6KA3	NM_004586	4759049	6197	D-003026-08	GGAGGTGAATTGCTGGATA	572
SGK						
SGK	NM_005627	5032090	6446	D-003027-01	TTAATGGTGGAGAGTTGTT	573
SGK	NM_005627	5032090	6446	D-003027-04	ATTAACTGGGATGATCTCA	574
SGK	NM_005627	25168262	6446	D-003027-05	GAAGAAAGCAATCCTGAAA	575
SGK	NM_005627	25168262	6446	D-003027-06	AAACACAGCTGAAATGTAC	576
TSC1						
TSC1	NM_000368	24475626	7248	D-003028-05	GAAGATGGCTATTCTGTGT	577
TSC1	NM_000368	24475626	7248	D-003028-06	TATGAAGGCTCGAGAGTTA	578
TSC1	NM_000368	24475626	7248	D-003028-07	CGACACGGCTGATAACTGA	579
TSC1	NM_000368	24475626	7248	D-003028-08	CGGCTGATGTTGTTAAATA	580
TSC2						
TSC2	NM_000548	10938006	7249	D-003029-05	GCATTAATCTCTTACCATA	581
TSC2	NM_000548	10938006	7249	D-003029-06	CCAATGTCCTCTTGTCTTT	582
TSC2	NM_000548	10938006	7249	D-003029-07	GGAGACACATCACCTACTT	583
TSC2	NM_000548	10938006	7249	D-003029-08	TCACCAGGCTCATCAAGAA	584
XPO1						
XPO1	NM_003400	8051634	7514	D-003030-05	GAAAGTCTCTGTCAAAATA	585
XPO1	NM_003400	8051634	7514	D-003030-06	GCAATAGGCTCCATTAGTG	586
XPO1	NM_003400	8051634	7514	D-003030-07	GGAACATGATCAACTTATA	587
XPO1	NM_003400	8051634	7514	D-003030-08	GGATACAGATTCCATAAAT	588

Table VII

Gene Name	Acc#	GI	L.L	Duplex #	Sequence	SI ID
ABL1						
ABL1	NM_007313	6382057	25	D-003100-05	GGAAATCAGTGACATAGTG	5
ABL1	NM_007313	6382057	25	D-003100-06	GGTCCACACTGCAATGTTT	5
ABL1	NM_007313	6382057	25	D-003100-07	GAAGGAAATCAGTGACATA	5
ABL1	NM_007313	6382057	25	D-003100-08	TCACTGAGTTCATGACCTA	5
ABL2						
ABL2	NM_007314	6382061	27	D-003101-05	GAAATGGAGCGAACAGATA	5
ABL2	NM_007314	6382061	27	D-003101-06	GAGCCAAATTTCCTATTAA	5
ABL2	NM_007314	6382061	27	D-003101-07	GTAATAAGCCTACAGTCTA	5
ABL2	NM_007314	6382061	27	D-003101-08	GGAGTGAAGTTCGCTCTAA	5
ACK1						
ACK1	NM_005781	8922074	10188	D-003102-05	AAACGCAAGTCGTGGATGA	5
ACK1	NM_005781	8922074	10188	D-003102-06	GCAAGTCGTGGATGAGTAA	5
ACK1	NM_005781	8922074	10188	D-003102-07	GAGCACTACCTCAGAATGA	5
ACK1	NM_005781	8922074	10188	D-003102-08	TCAGCAGCACCCACTATTA	6
ALK						
ALK	NM_004304	29029631	238	D-003103-05	GACAAGATCCTGCAGAATA	6
ALK	NM_004304	29029631	238	D-003103-06	GGAAGAGTCTGGCAGTTGA	6
ALK	NM_004304	29029631	238	D-003103-07	GCACGTGGCTCGGGACATT	6
ALK	NM_004304	29029631	238	D-003103-08	GAACTGCAGTGAAGGAACA	6
AXL						
AXL	NM_021913	21536465	558	D-003104-05	GGTCAGAGCTGGAGGATTT	6
AXL	NM_021913	21536465	558	D-003104-06	GAAAGAAGGAGACCCGTTA	6
AXL	NM_021913	21536465	558	D-003104-07	CCAAGAAGATCTACAATGG	6
AXL	NM_021913	21536465	558	D-003104-08	GGAACTGCATGCTGAATGA	6
BLK						
BLK	NM_001715	4502412	640	D-003105-05	GAGGATGCCTGCTGGATTT	6
BLK	NM_001715	4502412	640	D-003105-06	ACATGAAGGTGGCCATTAA	6
BLK	NM_001715	4502412	640	D-003105-07	GGTCAGCGCCCAAGACAAG	6
BLK	NM_001715	4502412	640	D-003105-08	GAAACTCGGGTCTGGACAA	6
BMX						
BMX	NM_001721	21359831	660	D-003106-05	AAACAAACCTTTCCTACTA	6
BMX	NM_001721	21359831	660	D-003106-06	GAAGGAGCATTTATGGTTA	6
BMX	NM_001721	21359831	660	D-003106-07	GAGAAGAGATTACCTTGTT	6
BMX	NM_001721	21359831	660	D-003106-08	GTAAGGCTGTGAATGATAA	6
BTK						
BTK	NM_000061	4557376	695	D-003107-05	GAACAGGAATGGAAGCTTA	6
BTK	NM_000061	4557376	695	D-003107-06	GCTATGGGCTGCCAAATTT	6
BTK	NM_000061	4557376	695	D-003107-07	GAAAGCAACTTACCATGGT	6
BTK	NM_000061	4557376	695	D-003107-08	GGTAAACGATCAAGGAGTT	6
C20orf64						
C20orf64	NM_033550	19923655	11285	D-003108-05	CAACTTAGCCAAGACAATT	6
C20orf64	NM_033550	19923655	11285	D-003108-06	GAAATTGAAGGCTCAGTGA	6
C20orf64	NM_033550	19923655	11285	D-003108-07	TGGAACAGCTGAACATTGT	6
C20orf64	NM_033550	19923655	11285	D-003108-08	GCTTCCAACTGCTTATATA	6
CSF1R						
CSF1R	NM_005211	27262658	1436	D-003109-05	GGAGAGCTCTGACGTTTGA	6
CSF1R	NM_005211	27262658	1436	D-003109-06	CAACAACGCTACCTTCCAA	6
CSF1R	NM_005211	27262658	1436	D-003109-07	CCACGCAGCTGCCTTACAA	6
CSF1R	NM_005211	27262658	1436	D-003109-08	GGAACAACCTGCAGTTTGG	6
CSK						

CSK	NM_004383	4758077	1445	D-003110-05	CAGAATGTATTGCCAAGTA	6
CSK	NM_004383	4758077	1445	D-003110-06	GAACAAAGTCGCCGTCAAG	6
CSK	NM_004383	4758077	1445	D-003110-07	GCGAGTGCCTTATCCAAGA	6
CSK	NM_004383	4758077	1445	D-003110-08	GGAGAAGGGCTACAAGATG	6
DDR1						
DDR1	NM_013994	7669484	780	D-003111-05	GGAGATGGAGTTTGAGTTT	6
DDR1	NM_013994	7669484	780	D-003111-06	CAGAGGCCCTGTCATCTTT	6
DDR1	NM_013994	7669484	780	D-003111-07	GCTGGTAGCTGTCAAGATC	6
DDR1	NM_013994	7669484	780	D-003111-08	TGAAAGAGGTGAAGATCAT	6
DDR2						
DDR2	NM_006182	5453813	4921	D-003112-05	GGTAAGAACTACACAATCA	6
DDR2	NM_006182	5453813	4921	D-003112-06	GAACGAGAGTGCCACCAAT	6
DDR2	NM_006182	5453813	4921	D-003112-07	ACACCAATCTGAAGTTTAT	6
DDR2	NM_006182	5453813	4921	D-003112-08	CAACAAGAATGCCAGGAAT	6
DKFZp761P1010						
DKFZp761P1010	NM_018423	8922178	55359	D-003113-05	CCTAGAAGCTGCCATTAAA	6
DKFZp761P1010	NM_018423	8922178	55359	D-003113-06	GATTAGGCCTGGCTTATGA	6
DKFZp761P1010	NM_018423	8922178	55359	D-003113-07	CCCAGTAGCTGCACACATA	6
DKFZp761P1010	NM_018423	8922178	55359	D-003113-08	GGTGGTACCTGAACTGTAT	6
EGFR						
EGFR	NM_005228	4885198	1956	D-003114-05	GAAGGAAACTGAATTCAAA	6
EGFR	NM_005228	4885198	1956	D-003114-06	GGAAATATGTACTACGAAA	6
EGFR	NM_005228	4885198	1956	D-003114-07	CCACAAAGCAGTGAATTTA	6
EGFR	NM_005228	4885198	1956	D-003114-08	GTAACAAGCTCACGCAGTT	6
EPHA1						
EPHA1	NM_005232	4885208	2041	D-003115-05	GACCAGAGCTTCACCATTC	6
EPHA1	NM_005232	4885208	2041	D-003115-06	GCAAGACTGTGGCCATTAA	6
EPHA1	NM_005232	4885208	2041	D-003115-07	GGGCGAACCTGACCTATGA	6
EPHA1	NM_005232	4885208	2041	D-003115-08	GATTGTAGCCGTCATCTTT	6
EPHA2						
EPHA2	NM_004431	4758277	1969	D-003116-05	GGAGGGATCTGGCAACTTG	6
EPHA2	NM_004431	4758277	1969	D-003116-06	GCAGCAAGGTGCACGAATT	6
EPHA2	NM_004431	4758277	1969	D-003116-07	GGAGAAGGATGGCGAGTTC	6
EPHA2	NM_004431	4758277	1969	D-003116-08	GAAGTTCACTACCGAGATC	6
EPHA3						
EPHA3	NM_005233	21361240	2042	D-003117-05	GATCGGACCTCCAGAAATA	6
EPHA3	NM_005233	21361240	2042	D-003117-06	GAACTCAGCTCAGAAGATT	6
EPHA3	NM_005233	21361240	2042	D-003117-07	GCAAGAGGCACAAATGTTA	6
EPHA3	NM_005233	21361240	2042	D-003117-08	GAGCATCAGTTTACAAAGA	6
EPHA4						
EPHA4	NM_004438	4758279	2043	D-003118-05	GGTCTGGGATGAAGTATTT	6
EPHA4	NM_004438	4758279	2043	D-003118-06	GAATGAAGTTACCTTATTG	6
EPHA4	NM_004438	4758279	2043	D-003118-07	GAACTTGGGTGGATAGCAA	6
EPHA4	NM_004438	4758279	2043	D-003118-08	GAGATTAAATTCACCTTGA	6
EPHA7						
EPHA7	NM_004440	4758281	2045	D-003119-05	GAAAAGAGATGTTGCAGTA	6
EPHA7	NM_004440	4758281	2045	D-003119-06	CTAGATGCCTCCTGTATTA	6
EPHA7	NM_004440	4758281	2045	D-003119-07	AGAAGAAGGTTATCGTTTA	6
EPHA7	NM_004440	4758281	2045	D-003119-08	TAGCAAAGCTGACCAAGAA	6
EPHA8						
EPHA8	NM_020526	18201903	2046	D-003120-05	GAAGATGCACTATCAGAAT	6

EPHA8	NM_020526	18201903	2046	D-003120-06	GAGAAGATGCACTATCAGA	
EPHA8	NM_020526	18201903	2046	D-003120-07	AACCTGATCTCCAGTGTGA	
EPHA8	NM_020526	18201903	2046	D-003120-08	TCTCAGACCTGGGCTATGT	
EPHB1						
EPHB1	NM_004441	21396502	2047	D-003121-05	GCGATAAGCTCCAGCATTA	
EPHB1	NM_004441	21396502	2047	D-003121-06	GAAACGGGCTTATAGCAAA	
EPHB1	NM_004441	21396502	2047	D-003121-07	GGATGAAGATCTACATTGA	
EPHB1	NM_004441	21396502	2047	D-003121-08	GCACGTCTCTGTCAACATC	
EPHB2						
EPHB2	NM_017449	17975764	2048	D-003122-05	ACTATGAGCTGCAGTACTA	
EPHB2	NM_017449	17975764	2048	D-003122-06	GTACAACGCCACAGCCATA	
EPHB2	NM_017449	17975764	2048	D-003122-07	GGAAAGCAATGACTGTTCT	
EPHB2	NM_017449	17975764	2048	D-003122-08	CGGACAAGCTGCAACACTA	
EPHB3						
EPHB3	NM_004443	17975767	2049	D-003123-05	GGTGTGATCTCCAATGTGA	
EPHB3	NM_004443	17975767	2049	D-003123-06	GGGATGACCTCCTGTACAA	
EPHB3	NM_004443	17975767	2049	D-003123-07	CAGAAGACCTGCTCCGTAT	
EPHB3	NM_004443	17975767	2049	D-003123-08	GAGATGAAGTACTTTGAGA	
EPHB4						
EPHB4	NM_004444	17975769	2050	D-003124-05	GGACAAACACGGACAGTAT	
EPHB4	NM_004444	17975769	2050	D-003124-06	GTACTAAGGTCTACATCGA	
EPHB4	NM_004444	17975769	2050	D-003124-07	GGAGAGAAGCAGAATATTC	
EPHB4	NM_004444	17975769	2050	D-003124-08	GCCAATAGCCACTCTAACA	
EPHB6						
EPHB6	NM_004445	4758291	2051	D-003125-05	GGAAGTCGATCCTGCTTAT	
EPHB6	NM_004445	4758291	2051	D-003125-06	GGACCAAGGTGGACACAAT	
EPHB6	NM_004445	4758291	2051	D-003125-07	TGTGGGAAGTGATGAGTTA	
EPHB6	NM_004445	4758291	2051	D-003125-08	CGGGAGACCTTCACCCTTT	
ERBB2						
ERBB2	NM_004448	4758297	2064	D-003126-05	GGACGAATTCTGCACAATG	
ERBB2	NM_004448	4758297	2064	D-003126-06	GACGAATTCTGCACAATGG	
ERBB2	NM_004448	4758297	2064	D-003126-07	CTACAACACAGACACGTTT	
ERBB2	NM_004448	4758297	2064	D-003126-08	AGACGAAGCATACGTGATG	
ERBB3						
ERBB3	NM_001982	4503596	2065	D-003127-05	AAGAGGATGTCAACGGTTA	
ERBB3	NM_001982	4503596	2065	D-003127-06	GAAGACTGCCAGACATTGA	
ERBB3	NM_001982	4503596	2065	D-003127-07	GACAAACACTGGTGCTGAT	
ERBB3	NM_001982	4503596	2065	D-003127-08	GCAGTGGATTCGAGAAGTG	
ERBB4						
ERBB4	NM_005235	4885214	2066	D-003128-05	GAGGAAAGATGCCAATTAA	
ERBB4	NM_005235	4885214	2066	D-003128-06	GCAGGAAACATCTATATTA	
ERBB4	NM_005235	4885214	2066	D-003128-07	GATCACAACTGCTGCTTAA	
ERBB4	NM_005235	4885214	2066	D-003128-08	CCTCAAAGATACCTAGTTA	
FER						
FER	NM_005246	4885230	2241	D-003129-05	GGAGTGACCTGAAGAATTC	
FER	NM_005246	4885230	2241	D-003129-06	TAAAGCAGATTCCCATTAA	
FER	NM_005246	4885230	2241	D-003129-07	GGAAAGTACTGTCCAAATG	
FER	NM_005246	4885230	2241	D-003129-08	GAACAACGGCTGCTAAAGA	
FES						
FES	NM_002005	13376997	2242	D-003130-05	CGAGGATCCTGAAGCAGTA	
FES	NM_002005	13376997	2242	D-003130-06	AGGAATACCTGGAGATTAG	
FES	NM_002005	13376997	2242	D-003130-07	CAACAGGAGCTCCGGAATG	
FES	NM_002005	13376997	2242	D-003130-08	GGTGTTGGGTGAGCAGATT	
FGFR1						
FGFR1	NM_000604	13186232	2260	D-003131-05	TAAGAAATGTCTCCTTTGA	
FGFR1	NM_000604	13186232	2260	D-003131-06	GAAGACTGCTGGAGTTAAT	

FGFR1	NM_000604	13186232	2260	D-003131-07	GATGGTCCCTTGTATGTCA	7
FGFR1	NM_000604	13186232	2260	D-003131-08	CTTAAGAAATGTCTCCTTT	7
FGFR2						
FGFR2	NM_000141	13186239	2263	D-003132-05	CCAAATCTCTCAACCAGAA	7
FGFR2	NM_000141	13186239	2263	D-003132-06	GAACAGTATTCACCTAGTT	7
FGFR2	NM_000141	13186239	2263	D-003132-07	GGCCAACACTGTCAAGTTT	7
FGFR2	NM_000141	13186239	2263	D-003132-08	GTGAAGATGTTGAAAGATG	7
FGFR3						
FGFR3	NM_000142	13112046	2261	D-003133-05	TGTCGGACCTGGTGTCTGA	7
FGFR3	NM_000142	13112046	2261	D-003133-06	GCATCAAGCTGCGGCATCA	7
FGFR3	NM_000142	13112046	2261	D-003133-07	GGACGGCACACCCTACGTT	7
FGFR3	NM_000142	13112046	2261	D-003133-08	TGCACAACCTCGACTACTA	7
FGFR4						
FGFR4	NM_002011	13112051	2264	D-003134-05	GCACTGGAGTCTCGTGATG	7
FGFR4	NM_002011	13112051	2264	D-003134-06	CATAGGGACCTCTCGAATA	7
FGFR4	NM_002011	13112051	2264	D-003134-07	ATACGGACATCATCCTGTA	7
FGFR4	NM_002011	13112051	2264	D-003134-08	ATAGGGACCTCTCGAATAG	7
FGR						
FGR	NM_005248	4885234	2268	D-003135-05	GCGATCATGTGAAGCATTA	7
FGR	NM_005248	4885234	2268	D-003135-06	TCACTGAGCTCATCACCAA	7
FGR	NM_005248	4885234	2268	D-003135-07	GAAGAGTGGTACTTTGGAA	7
FGR	NM_005248	4885234	2268	D-003135-08	CCCAGAAGCTGCCCTCTTT	7
FLT1						
FLT1	NM_002019	4503748	2321	D-003136-05	GAGCAAACGTGACTTATTT	7
FLT1	NM_002019	4503748	2321	D-003136-06	CCAAATGGGTTTCATGTTA	7
FLT1	NM_002019	4503748	2321	D-003136-07	CAACAAGGATGCAGCACTA	7
FLT1	NM_002019	4503748	2321	D-003136-08	GGACGTAACTGAAGAGGAT	7
FLT3						
FLT3	NM_004119	4758395	2322	D-003137-05	GAAGGCATCTACACCATTA	7
FLT3	NM_004119	4758395	2322	D-003137-06	GAAGGAGTCTGGAATAGAA	7
FLT3	NM_004119	4758395	2322	D-003137-07	GAATTTAAGTCGTGTGTTC	7
FLT3	NM_004119	4758395	2322	D-003137-08	GGAATTCATTTCACTCTGA	7
FLT4						
FLT4	NM_002020	4503752	2324	D-003138-05	GCAAGAACGTGCATCTGTT	7
FLT4	NM_002020	4503752	2324	D-003138-06	GCGAATACCTGTCCTACGA	7
FLT4	NM_002020	4503752	2324	D-003138-07	GAAGACATTTGAGGAATTC	7
FLT4	NM_002020	4503752	2324	D-003138-08	GAGCAGCCATTCATCAACA	7
FRK						
FRK	NM_002031	4503786	2444	D-003139-05	GAAACAGACTCTTCATATT	7
FRK	NM_002031	4503786	2444	D-003139-06	GAACAATACCACTCCAGTA	7
FRK	NM_002031	4503786	2444	D-003139-07	CAAGACCGGTTCCTTTCTA	7
FRK	NM_002031	4503786	2444	D-003139-08	GCAAGAATATCTCCAAAAT	7
FYN						
FYN	NM_002037	23510344	2534	D-003140-05	GGAATGGACTCATATGCAA	7
FYN	NM_002037	23510344	2534	D-003140-06	GCAGAAGAGTGGTACTTTG	7
FYN	NM_002037	23510344	2534	D-003140-07	CAAAGGAAGTTTACTGGAT	7
FYN	NM_002037	23510344	2534	D-003140-08	GAAGAGTGGTACTTTGGAA	7
HCK						
HCK	NM_002110	4504356	3055	D-003141-05	GAGATACCGTGAAACATTA	7
HCK	NM_002110	4504356	3055	D-003141-06	GCAGGGAGATACCGTGAAA	7
HCK	NM_002110	4504356	3055	D-003141-07	CATCGTGGTTGCCCTGTAT	7
HCK	NM_002110	4504356	3055	D-003141-08	TGTGTAAGATTGCTGACTT	7
ITK						
ITK	NM_005546	21614549	3702	D-003144-05	CAAATAATCTGGAAACCTA	7
ITK	NM_005546	21614549	3702	D-003144-06	GAAGAAACGAGGAATAATA	7
ITK	NM_005546	21614549	3702	D-003144-07	GAAACTCTCTCATCCCAAA	7

ITK	NM_005546	21614549	3702	D-003144-08	GGAATGGGCATGAAGGATA	7
JAK1						
JAK1	NM_002227	4504802	3716	D-003145-05	CCACATAGCTGATCTGAAA	7
JAK1	NM_002227	4504802	3716	D-003145-06	TGAAATCACTCACATTGTA	7
JAK1	NM_002227	4504802	3716	D-003145-07	TAAGGAACCTCTATCATGA	7
JAK1	NM_002227	4504802	3716	D-003145-08	GCAGGTGGCTGTTAAATCT	7
JAK2						
JAK2	NM_004972	13325062	3717	D-003146-05	GCAAATAGATCCAGTTCTT	7
JAK2	NM_004972	13325062	3717	D-003146-06	GAGCAAAGATCCAAGACTA	7
JAK2	NM_004972	13325062	3717	D-003146-07	GCCAGAAACTTGAAACTTA	7
JAK2	NM_004972	13325062	3717	D-003146-08	GTACAGATTTCGCAGATTT	7
JAK3						
JAK3	NM_000215	4557680	3718	D-003147-05	GCGCCTATCTTTCTCCTTT	7
JAK3	NM_000215	4557680	3718	D-003147-06	CCAGAAATCGTAGACATTA	7
JAK3	NM_000215	4557680	3718	D-003147-07	CCTCATCTCTTCAGACTAT	7
JAK3	NM_000215	4557680	3718	D-003147-08	TGTACGAGCTCTTCACCTA	7
KDR						
KDR	NM_002253	11321596	3791	D-003148-05	GGAAATCTCTTGCAAGCTA	7
KDR	NM_002253	11321596	3791	D-003148-06	GATTACAGATCTCCATTTA	7
KDR	NM_002253	11321596	3791	D-003148-07	GCAGACAGATCTACGTTTG	7
KDR	NM_002253	11321596	3791	D-003148-08	GCGATGGCCTCTTCTGTAA	7
KIAA1079						
KIAA1079	NM_014916	7662475	22853	D-003149-05	GAAATTCTCTCAACTGATG	7
KIAA1079	NM_014916	7662475	22853	D-003149-06	GCAGAGGTCTTCACACTTT	7
KIAA1079	NM_014916	7662475	22853	D-003149-07	TAAATGATCTTCAGACAGA	7
KIAA1079	NM_014916	7662475	22853	D-003149-08	GAGCAGCCCTACTCTGATA	7
KIT						
KIT	NM_000222	4557694	3815	D-003150-05	AAACACGGCTTAAGCAATT	7
KIT	NM_000222	4557694	3815	D-003150-06	GAACAGAACCTTCACTGAT	7
KIT	NM_000222	4557694	3815	D-003150-07	GGGAAGCCCTCATGTCTGA	7
KIT	NM_000222	4557694	3815	D-003150-08	GCAATTCCATTTATGTGTT	7
LCK						
LCK	NM_005356	20428651	3932	D-003151-05	GAACTGCCATTATCCCATA	7
LCK	NM_005356	20428651	3932	D-003151-06	GAGAGGTGGTGAAACATTA	7
LCK	NM_005356	20428651	3932	D-003151-07	GGGCCAAGTTTCCCATTAA	7
LCK	NM_005356	20428651	3932	D-003151-08	GCACGCTGCTCATCCGAAA	7
LTK						
LTK	NM_002344	4505044	4058	D-003152-05	TGAATTCACTCCTGCCAAT	7
LTK	NM_002344	4505044	4058	D-003152-06	GTGGCAACCTCAACACTGA	7
LTK	NM_002344	4505044	4058	D-003152-07	GGAGCTAGCTGTGGATAAC	7
LTK	NM_002344	4505044	4058	D-003152-08	GCAAGTTTCGCCATCAGAA	7
LYN						
LYN	NM_002350	4505054	4067	D-003153-05	GCAGATGGCTTGTGCAGAA	7
LYN	NM_002350	4505054	4067	D-003153-06	GGAGAAGGCTTGTATTAGT	7
LYN	NM_002350	4505054	4067	D-003153-07	GATGAGCTCTATGACATTA	7
LYN	NM_002350	4505054	4067	D-003153-08	GGTGCTAAGTTCCCTATTA	7
MATK						
MATK	NM_002378	21450841	4145	D-003154-05	TGAAGAATATCAAGTGTGA	7
MATK	NM_002378	21450841	4145	D-003154-06	CCGCTCAGCTCCTGCAGTT	7
MATK	NM_002378	21450841	4145	D-003154-07	TACTGAACCTGCAGCATTT	7
MATK	NM_002378	21450841	4145	D-003154-08	TGGGAGGTCTTCTCATATG	8
MERTK						
MERTK	NM_006343	5453737	10461	D-003155-05	GAACTTACCTTACATAGCT	8
MERTK	NM_006343	5453737	10461	D-003155-06	GGACCTGCATACTTACTTA	8
MERTK	NM_006343	5453737	10461	D-003155-07	TGACAGGAATCTTCTAATT	8
MERTK	NM_006343	5453737	10461	D-003155-08	GGTAATGGCTCAGTCATGA	8

MET						
MET	NM_000245	4557746	4233	D-003156-05	GAAAGAACCTCTCAACATT	8
MET	NM_000245	4557746	4233	D-003156-06	GGACAAGGCTGACCATATG	8
MET	NM_000245	4557746	4233	D-003156-07	CCAATGACCTGCTGAAATT	8
MET	NM_000245	4557746	4233	D-003156-08	GAGCATACATTAAACCAAA	8
MST1R						
MST1R	NM_002447	4505264	4486	D-003157-05	GGATGGAGCTGCTGGCTTT	8
MST1R	NM_002447	4505264	4486	D-003157-06	CTGCAGACCTATAGATTTA	8
MST1R	NM_002447	4505264	4486	D-003157-07	GCACCTGTCTCACTCTTGA	8
MST1R	NM_002447	4505264	4486	D-003157-08	GAAAGAGTCCATCCAGCTA	8
MUSK						
MUSK	NM_005592	5031926	4593	D-003158-05	GAAGAAGCCTCGGCAGATA	8
MUSK	NM_005592	5031926	4593	D-003158-06	GTAATAATCTCCATCATGT	8
MUSK	NM_005592	5031926	4593	D-003158-07	GGAATGAACTGAAAGTAGT	8
MUSK	NM_005592	5031926	4593	D-003158-08	GAGATTTCCTGGACTAGAA	8
NTRK1						
NTRK1	NM_002529	4585711	4914	D-003159-05	GGACAACCCTTTCGAGTTC	8
NTRK1	NM_002529	4585711	4914	D-003159-06	CCAGTGACCTCAACAGGAA	8
NTRK1	NM_002529	4585711	4914	D-003159-07	CCACAATACTTCAGTGATG	8
NTRK1	NM_002529	4585711	4914	D-003159-08	GAAGAGTGGTCTCCGTTTC	8
NTRK2						
NTRK2	NM_006180	21361305	4915	D-003160-05	GAACAGAAGTAATGAAATC	8
NTRK2	NM_006180	21361305	4915	D-003160-06	GTAATGCTGTTTCTGCTTA	8
NTRK2	NM_006180	21361305	4915	D-003160-07	GCAAGACACTCCAAGTTTG	8
NTRK2	NM_006180	21361305	4915	D-003160-08	GAAAGTCTATCACATTATC	8
NTRK3						
NTRK3	NM_002530	4505474	4916	D-003161-05	GAGCGAATCTGCTAGTGAA	8
NTRK3	NM_002530	4505474	4916	D-003161-06	GAAGTTCACTACAGAGAGT	8
NTRK3	NM_002530	4505474	4916	D-003161-07	GGTCGACGGTCCAAATTTG	8
NTRK3	NM_002530	4505474	4916	D-003161-08	GAATATCACTTCCATACAC	8
PDGFRA						
PDGFRA	NM_006206	15451787	5156	D-003162-05	GAAACTTCCTGGACTATTT	8
PDGFRA	NM_006206	15451787	5156	D-003162-06	GAGATATGGTCAACTATTT	8
PDGFRA	NM_006206	15451787	5156	D-003162-07	GCACGCCGCTTCCTGATAT	8
PDGFRA	NM_006206	15451787	5156	D-003162-08	CATCAGAGCTGGATCTAGA	8
PDGFRB						
PDGFRB	NM_002609	15451788	5159	D-003163-05	GAAAGGAGACGTCAAATAT	8
PDGFRB	NM_002609	15451788	5159	D-003163-06	GGAATGAGGTGGTCAACTT	8
PDGFRB	NM_002609	15451788	5159	D-003163-07	CAACGAGTCTCCAGTGCTA	8
PDGFRB	NM_002609	15451788	5159	D-003163-08	GAGAGGACCTGCCGAGCAA	8
PTK2						
PTK2	NM_005607	27886592	5747	D-003164-05	GAAGTTGGGTTGTCTAGAA	8
PTK2	NM_005607	27886592	5747	D-003164-06	GAAGAACAATGATGTAATC	8
PTK2	NM_005607	27886592	5747	D-003164-07	GGAAATTGCTTTGAAGTTG	8
PTK2	NM_005607	27886592	5747	D-003164-08	GGTTCAAGCTGGATTATTT	8
PTK2B						
PTK2B	NM_004103	27886583	2185	D-003165-05	GAACATGGCTGACCTCATA	8
PTK2B	NM_004103	27886583	2185	D-003165-06	GGACCACGCTGCTCTATTT	8
PTK2B	NM_004103	27886583	2185	D-003165-07	GGACGAGGACTATTACAAA	8
PTK2B	NM_004103	27886583	2185	D-003165-08	TGGCAGAGCTCATCAACAA	8
PTK6						
PTK6	NM_005975	27886594	5753	D-003166-05	GAGAAAGTCCTGCCCGTTT	8
PTK6	NM_005975	27886594	5753	D-003166-06	TGAAGAAGCTGCGGCACAA	8
PTK6	NM_005975	27886594	5753	D-003166-07	CCGCGACTCTGATGAGAAA	8
PTK6	NM_005975	27886594	5753	D-003166-08	TGCCCGAGCTTGTGAACTA	8
PTK7						

PTK7	NM_002821	27886610	5754	D-003167-05	GAGAGAAGCCCACTATTAA	8
PTK7	NM_002821	27886610	5754	D-003167-06	CGAGAGAAGCCCACTATTA	8
PTK7	NM_002821	27886610	5754	D-003167-07	GGAGGGAGTTGGAGATGTT	8
PTK7	NM_002821	27886610	5754	D-003167-08	GAAGACATGCCGCTATTTG	8
PTK9						
PTK9	NM_002822	4506274	5756	D-003168-05	GAAGAACTACGACAGATTA	8
PTK9	NM_002822	4506274	5756	D-003168-09	GAAGGAGACTATTTAGAGT	8
PTK9	NM_002822	4506274	5756	D-003168-10	GAGCGGATGCTGTATTCTA	8
PTK9	NM_002822	4506274	5756	D-003168-11	CTGCAGACTTCCTTTATGA	8
PTK9L						
PTK9L	NM_007284	31543446	11344	D-003169-05	AGAGAGAGCTCCAGCAGAT	8
PTK9L	NM_007284	31543446	11344	D-003169-06	TTAACGAGGTGAAGACAGA	8
PTK9L	NM_007284	31543446	11344	D-003169-07	ACACAGAGCCCACGGATGT	8
PTK9L	NM_007284	31543446	11344	D-003169-08	GCTGGGATCAGGACTATGA	8
RET						
RET	NM_000323	21536316	5979	D-003170-05	GCAAAGACCTGGAGAAGAT	8
RET	NM_000323	21536316	5979	D-003170-06	GCACACGGCTGCATGAGAA	8
RET	NM_000323	21536316	5979	D-003170-07	GAACTGGCCTGGAGAGAGT	8
RET	NM_000323	21536316	5979	D-003170-08	TTAAATGGATGGCAATTGA	8
ROR1						
ROR1	NM_005012	4826867	4919	D-003171-05	GCAAGCATCTTTACTAGGA	8
ROR1	NM_005012	4826867	4919	D-003171-06	GAGCAAGGCTAAAGAGCTA	8
ROR1	NM_005012	4826867	4919	D-003171-07	GAGAGCAACTTCATGTAAA	8
ROR1	NM_005012	4826867	4919	D-003171-08	GAGAATGTCCTGTGTCAAA	8
ROR2						
ROR2	NM_004560	19743897	4920	D-003172-05	GGAACTCGCTGCTGCCTAT	8
ROR2	NM_004560	19743897	4920	D-003172-06	GCAGGTGCCTCCTCAGATG	8
ROR2	NM_004560	19743897	4920	D-003172-07	GCAATGTGCTAGTGTACGA	8
ROR2	NM_004560	19743897	4920	D-003172-08	GAAGACAGAATATGGTTCA	8
ROS1						
ROS1	NM_002944	19924164	6098	D-003173-05	GAGGAGACCTTCTTACTTA	8
ROS1	NM_002944	19924164	6098	D-003173-06	TTACAGAGGTTCAGGATTA	8
ROS1	NM_002944	19924164	6098	D-003173-07	GAACAAACGTAAGCATGAA	8
ROS1	NM_002944	19924164	6098	D-003173-08	GAAAGAGCACTTCAAATAA	8
RYK						
RYK	NM_002958	11863158	6259	D-003174-05	GAAAGATGGTTACCGAATA	8
RYK	NM_002958	11863158	6259	D-003174-06	CAAAGTAGATTCTGAAGTT	8
RYK	NM_002958	11863158	6259	D-003174-07	TCACTACGCTCTATCCTTT	8
RYK	NM_002958	11863158	6259	D-003174-08	GGTGAAGGATATAGCAATA	8
SRC						
SRC	NM_005417	21361210	6714	D-003175-05	GAGAACCTGGTGTGCAAAG	8
SRC	NM_005417	21361210	6714	D-003175-09	GAGAGAACCTGGTGTGCAA	8
SRC	NM_005417	21361210	6714	D-003175-10	GGAGTTTGCTGGACTTTCT	8
SRC	NM_005417	21361210	6714	D-003175-11	GAAAGTGAGACCACGAAAG	8
SYK						
SYK	NM_003177	21361552	6850	D-003176-05	GGAATAATCTCAAGAATCA	8
SYK	NM_003177	21361552	6850	D-003176-06	GAACTGGGCTCTGGTAATT	8
SYK	NM_003177	21361552	6850	D-003176-07	GGAAGAATCTGAGCAAATT	8
SYK	NM_003177	21361552	6850	D-003176-08	GAACAGACATGTCAAGGAT	8
TEC						
TEC	NM_003215	4507428	7006	D-003177-05	GAAATTGTCTAGTAAGTGA	8
TEC	NM_003215	4507428	7006	D-003177-06	CACCTGAAGTGTTTAATTA	8
TEC	NM_003215	4507428	7006	D-003177-07	GTACAAAGTCGCAATCAAA	8
TEC	NM_003215	4507428	7006	D-003177-08	TGGAGGAGATTCTTATTAA	8
TEK						
TEK	NM_000459	4557868	7010	D-003178-05	GAAAGAATATGCCTCCAAA	8

TEK	NM_000459	4557868	7010	D-003178-06	GGAATGACATCAAATTTCA	8
TEK	NM_000459	4557868	7010	D-003178-07	TGAAGTACCTGATATTCTA	8
TEK	NM_000459	4557868	7010	D-003178-08	CGAAAGACCTACGTGAATA	8
TIE						
TIE	NM_005424	4885630	7075	D-003179-05	GAGAGGAGGTTTATGTGAA	8
TIE	NM_005424	4885630	7075	D-003179-06	GGGACAGCCTCTACCCTTA	8
TIE	NM_005424	4885630	7075	D-003179-07	GAAGTTCTGTGCAAATTGG	8
TIE	NM_005424	4885630	7075	D-003179-08	CAACATGGCCTCAGAACTG	9
TNK1						
TNK1	NM_003985	4507610	8711	D-003180-05	GTTCTGGGCCTAAGTCTAA	9
TNK1	NM_003985	4507610	8711	D-003180-06	GAACTGGGTCTACAAGATC	9
TNK1	NM_003985	4507610	8711	D-003180-07	CGAGAGGTATCGGTCATGA	9
TNK1	NM_003985	4507610	8711	D-003180-08	GGCGCATCCTGGAGCATTA	9
TXK						
TXK	NM_003328	4507742	7294	D-003181-05	GAACATCTATTGAGACAAG	9
TXK	NM_003328	4507742	7294	D-003181-06	TCAAGGCACTTTATGATTT	9
TXK	NM_003328	4507742	7294	D-003181-07	GGAGAGGAATGGCTATATT	9
TXK	NM_003328	4507742	7294	D-003181-08	GGATATATGTGAAGGAATG	9
TYK2						
TYK2	NM_003331	4507748	7297	D-003182-05	GAGGAGATCCACCACTTTA	9
TYK2	NM_003331	4507748	7297	D-003182-06	GCATCCACATTGCACATAA	9
TYK2	NM_003331	4507748	7297	D-003182-07	TCAAATACCTAGCCACACT	9
TYK2	NM_003331	4507748	7297	D-003182-08	CAATCTTGCTGACGTCTTG	9
TYRO3						
TYRO3	NM_006293	27597077	7301	D-003183-05	GGTAGAAGGTGTGCCATTT	9
TYRO3	NM_006293	27597077	7301	D-003183-06	ACGCTGAGATTTACAACTA	9
TYRO3	NM_006293	27597077	7301	D-003183-07	GGATGGCTCCTTTGTGAAA	9
TYRO3	NM_006293	27597077	7301	D-003183-08	GAGAGGAACTACGAAGATC	9
YES1						
YES1	NM_005433	21071041	7525	D-003184-05	GAAGGACCCTGATGAAAGA	9
YES1	NM_005433	21071041	7525	D-003184-06	TAAGAAGGGTGAAAGATTT	9
YES1	NM_005433	21071041	7525	D-003184-07	TCAAGAAGCTCAGATAATG	9
YES1	NM_005433	21071041	7525	D-003184-08	CAGAATCCCTCCATGAATT	9

Table VIII

Gene Name	Acc#	GI	Locus Link	Duplex #	Full Sequence	SEQ. ID NO.
APC2						
APC2	NM_013366	7549800	29882	D-003200-05	GCAAGGACCTCTTCATCAA	921
APC2	NM_013366	7549800	29882	D-003200-06	GAGAAGAAGTCCACACTAT	922
APC2	NM_013366	7549800	29882	D-003200-07	GGAATGCCATCTCCCAATG	923
APC2	NM_013366	7549800	29882	D-003200-09	CAACACGTGTGACATCATC	924
ATM						
ATM	NM_000051	20336202	472	D-003201-05	GCAAGCAGCTGAAACAAAT	925
ATM	NM_000051	20336202	472	D-003201-06	GAATGTTGCTTTCTGAATT	926
ATM	NM_000051	20336202	472	D-003201-07	GACCTGAAGTCTTATTTAA	927
ATM	NM_000051	20336202	472	D-003201-08	AGACAGAATTCCCAAATAA	928
ATR						
ATR	NM_001184	20143978	545	D-003202-05	GAACAACACTGCTGGTTTG	929
ATR	NM_001184	20143978	545	D-003202-06	GAAGTCATCTGTTCATTAT	930
ATR	NM_001184	20143978	545	D-003202-07	GAAATAAGGTAGACTCAAT	931
ATR	NM_001184	20143978	545	D-003202-08	CAACATAAATCCAAGAAGA	932
BTAK						

BTAK	NM_003600	3213196	6790	D-003545-04	CAAAGAATCAGCTAGCAAA	933
BTAK	NM_003600	3213196	6790	D-003203-05	GAAGAGAGTTATTCATAGA	934
BTAK	NM_003600	3213196	6790	D-003203-07	CAAATGCCCTGTCTTACTG	935
STK6	NM_003600	3213196	6790	D-003203-09	TCTCGTGACTCAGCAAATT	936
CCNA1						
CCNA1	NM_003914	16306528	8900	D-003204-05	GAACCTGGCTAAGTACGTA	937
CCNA1	NM_003914	16306528	8900	D-003204-06	GCAGATCCATTCTTGAAAT	938
CCNA1	NM_003914	16306528	8900	D-003204-07	TCACAAGAATCAGGTGTTA	939
CCNA1	NM_003914	16306528	8900	D-003204-08	CATAAAGCGTACCTTGATA	940
CCNA2						
CCNA2	NM_001237	16950653	890	D-003205-05	GCTGTGAACTACATTGATA	941
CCNA2	NM_001237	16950653	890	D-003205-06	GATGATACCTACACCAAGA	942
CCNA2	NM_001237	16950653	890	D-003205-07	GCTGTTAGCCTCAAAGTTT	943
CCNA2	NM_001237	16950653	890	D-003205-08	AAGCTGGCCTGAATCATTA	944
CCNB1						
CCNB1	NM_031966	14327895	891	D-003206-05	CAACATTACCTGTCATATA	945
CCNB1	NM_031966	14327895	891	D-003206-06	CCAAATACCTGATGGAACT	946
CCNB1	NM_031966	14327895	891	D-003206-07	GAAATGTACCCTCCAGAAA	947
CCNB1	NM_031966	14327895	891	D-003206-08	GCACCTGGCTAAGAATGTA	948
CCNB2						
CCNB2	NM_004701	10938017	9133	D-003207-05	CAACAAATGTCAACAAACA	949
CCNB2	NM_004701	10938017	9133	D-003207-06	GCAGCAAACTCCTGAAGAT	950
CCNB2	NM_004701	10938017	9133	D-003207-07	CCAGTGATTTGGAGAATAT	951
CCNB2	NM_004701	10938017	9133	D-003207-08	GTGACTACGTTAAGGATAT	952
CCNB3						
CCNB3	NM_033031	14719419	85417	D-003208-05	TGAACAAACTGCTGACTTT	953
CCNB3	NM_033031	14719419	85417	D-003208-06	GCTAGCTGCTGCCTCCTTA	954
CCNB3	NM_033031	14719419	85417	D-003208-07	CAACTCACCTCGTGTGGAT	955
CCNB3	NM_033031	14719419	85417	D-003208-08	GTGGATCTCTACCTAATGA	956
CCNC						
CCNC	NM_005190	7382485	892	D-003209-05	GCAGAGCTCCCACTATTTG	957
CCNC	NM_005190	7382485	892	D-003209-06	GGAGTAGTTTCAAATACAA	958
CCNC	NM_005190	7382485	892	D-003209-07	GACCTTTGCTCCAGTATGT	959
CCNC	NM_005190	7382485	892	D-003209-08	GAGATTCTATGCCAGGTAT	960
CCND1						
CCND1	NM_053056	16950654	595	D-003210-05	TGAACAAGCTCAAGTGGAA	961
CCND1	NM_053056	16950654	595	D-003210-06	CCAGAGTGATCAAGTGTGA	962
CCND1	NM_053056	16950654	595	D-003210-07	GTTCGTGGCCTCTAAGATG	963
CCND1	NM_053056	16950654	595	D-003210-08	CCGAGAAGCTGTGCATCTA	964
CCND2						
CCND2	NM_001759	16950656	894	D-003211-06	TGAATTACCTGGACCGTTT	965
CCND2	NM_001759	16950656	894	D-003211-07	CGGAGAAGCTGTGCATTTA	966
CCND2	NM_001759	16950656	894	D-003211-08	CTACAGACGTGCGGGATAT	967
CCND2	NM_001759	16950656	894	D-003211-09	CAACACAGACGTGGATTGT	968
CCND3						
CCND3	NM_001760	16950657	896	D-003212-05	GGACCTGGCTGCTGTGATT	969
CCND3	NM_001760	16950657	896	D-003212-06	GATTATACCTTTGCCATGT	970
CCND3	NM_001760	16950657	896	D-003212-07	GACCAGCACTCCTACAGAT	971
CCND3	NM_001760	16950657	896	D-003212-08	TGCGGAAGATGCTGGCTTA	972
CCNE1						
CCNE1	NM_001238	17318558	898	D-003213-05	GTACTGAGCTGGGCAAATA	973
CCNE1	NM_001238	17318558	898	D-003213-06	GGAAATCTATCCTCCAAAG	974
CCNE1	NM_001238	17318558	898	D-003213-07	GGAGGTGTGTGAAGTCTAT	975
CCNE1	NM_001238	17318558	898	D-003213-08	CTAAATGACTTACATGAAG	976
CCNE2						
CCNE2	NM_057749	17318564	9134	D-003214-05	GGATGGAACTCATTATATT	977

CCNE2	NM_057749	17318564	9134	D-003214-06	GCAGATATGTTCATGACAA	978
CCNE2	NM_057749	17318564	9134	D-003214-07	CATAATATCCAGACACATA	979
CCNE2	NM_057749	17318564	9134	D-003214-08	TAAGAAAGCCTCAGGTTTG	980
CCNF						
CCNF	NM_001761	4502620	899	D-003215-05	TCACAAAGCATCCATATTG	981
CCNF	NM_001761	4502620	899	D-003215-06	GAAGTCATGTTTACAGTGT	982
CCNF	NM_001761	4502620	899	D-003215-07	TAGCCTACCTCTACAATGA	983
CCNF	NM_001761	4502620	899	D-003215-08	GCACCCGGTTTATCAGTAA	984
CCNG1						
CCNG1	NM_004060	8670528	900	D-003216-05	GATAATGGCCTCAGAATGA	985
CCNG1	NM_004060	8670528	900	D-003216-06	GCACGGCAATTGAAGCATA	986
CCNG1	NM_004060	8670528	900	D-003216-07	GGAATAGAATGTCTTCAGA	987
CCNG1	NM_004060	8670528	900	D-003216-08	TAACTCACCTTCCAACAAT	988
CCNG2						
CCNG2	NM_004354	4757935	901	D-003217-05	GGAGAGAGTTGGTTTCTAA	989
CCNG2	NM_004354	4757935	901	D-003217-06	GGTGAAACCTAAACATTTG	990
CCNG2	NM_004354	4757935	901	D-003217-07	GAAATACTGAGCCTTGATA	991
CCNG2	NM_004354	4757935	901	D-003217-08	TGCCAAAGTTGAAGATTTA	992
CCNH						
CCNH	NM_001239	17738313	902	D-003218-05	GCTGATGACTTTCTTAATA	993
CCNH	NM_001239	17738313	902	D-003218-06	CAACTTAATTTCCACCTTA	994
CCNH	NM_001239	17738313	902	D-003218-07	ATACACACCTTCCCAAATT	995
CCNH	NM_001239	17738313	902	D-003218-08	GCTATGAAGATGATGATTA	996
CCNI						
CCNI	NM_006835	17738314	10983	D-003219-05	GCAAGCAGACCTCTACTAA	997
CCNI	NM_006835	17738314	10983	D-003219-07	TGAGAGAATTCCAGTACTA	998
CCNI	NM_006835	17738314	10983	D-003219-08	GGAATCAAACGGCTCTATA	999
CCNI	NM_006835	17738314	10983	D-003219-09	GAATTGGGATCTTCACACA	1000
CCNT1						
CCNT1	NM_001240	17978465	904	D-003220-05	TATCAACACTGCTATAGTA	1001
CCNT1	NM_001240	17978465	904	D-003220-06	GAACAAACGTCCTGGTGAT	1002
CCNT1	NM_001240	17978465	904	D-003220-07	GCACAAGACTCACCCATCT	1003
CCNT1	NM_001240	17978465	904	D-003220-08	GCACAGACTTCTTACTTCA	1004
CCNT2A						
CCNT2A	NM_001241	17978467	905	D-003221-05	GCACAGACATCCTATTTCA	1005
CCNT2A	NM_001241	17978467	905	D-003221-06	GCAGGGACCTTCTATATCA	1006
CCNT2A	NM_001241	17978467	905	D-003221-07	GAACAGCTATATTCACAGA	1007
CCNT2A	NM_001241	17978467	905	D-003221-09	TTATATAGCTGCCCAGGTA	1008
CCNT2B						
CCNT2B	NM_058241	17978468	905	D-003222-05	GCACAGACATCCTATTTCA	1009
CCNT2B	NM_058241	17978468	905	D-003222-06	GCAGGGACCTTCTATATCA	1010
CCNT2B	NM_058241	17978468	905	D-003222-07	GAACAGCTATATTCACAGA	1011
CCNT2B	NM_058241	17978468	905	D-003222-08	GGTGAAATGTACCCAGTTA	1012
CDC16						
CDC16	NM_003903	14110370	8881	D-003223-05	GTAGATGGCTTGCAAGAGA	1013
CDC16	NM_003903	14110370	8881	D-003223-06	TAAAGTAGCTTCACTCTCT	1014
CDC16	NM_003903	14110370	8881	D-003223-07	GCTACAAGCTTACTTCTGT	1015
CDC16	NM_003903	14110370	8881	D-003223-08	TGGAAGAGCCCATCAATAA	1016
CDC2						
CDC2	NM_033379	27886643	983	D-003552-01	GTACAGATCTCCAGAAGTA	1017
CDC2	NM_033379	27886643	983	D-003552-02	GATCAACTCTTCAGGATTT	1018
CDC2	NM_033379	27886643	983	D-003552-03	GGTTATATCTCATCTTTGA	1019
CDC2	NM_033379	27886643	983	D-003552-04	GAACTTCGTCATCCAAATA	1020
CDC20						
CDC20	NM_001255	4557436	991	D-003225-05	GGGAATATATATCCTCTGT	1021
CDC20	NM_001255	4557436	991	D-003225-06	GAAACGGCTTCGAAATATG	1022

CDC20	NM_001255	4557436	991	D-003225-07	GAAGACCTGCCGTTACATT	1023
CDC20	NM_001255	4557436	991	D-003225-08	CACCAGTGATCGACACATT	1024
CDC25A						
CDC25A	NM_001789	4502704	993	D-003226-05	GAAATTATGGCATCTGTTT	1025
CDC25A	NM_001789	4502704	993	D-003226-06	TACAAGGAGTTCTTTATGA	1026
CDC25A	NM_001789	4502704	993	D-003226-07	CCACGAGGACTTTAAAGAA	1027
CDC25A	NM_001789	4502704	993	D-003226-08	TGGGAAACATCAGGATTTA	1028
CDC25B						
CDC25B	NM_004358	11641416	994	D-003227-05	GCAGATACCCCTATGAATA	1029
CDC25B	NM_004358	11641416	994	D-003227-06	CTAGGTCGCTTCTCTCTGA	1030
CDC25B	NM_004358	11641416	994	D-003227-07	GAGAGCTGATTGGAGATTA	1031
CDC25B	NM_004358	11641416	994	D-003227-08	AAAAGGACCTCGTCATGTA	1032
CDC25C						
CDC25C	NM_001790	12408659	995	D-003228-05	GAGCAGAAGTGGCCTATAT	1033
CDC25C	NM_001790	12408659	995	D-003228-06	CAGAAGAGATTTCAGATGA	1034
CDC25C	NM_001790	12408659	995	D-003228-07	CCAGGGAGCCTTAAACTTA	1035
CDC25C	NM_001790	12408659	995	D-003228-08	GAAACTTGGTGGACAGTGA	1036
CDC27						
CDC27	NM_001256	16554576	996	D-003229-06	CATGCAAGCTGAAAGAATA	1037
CDC27	NM_001256	16554576	996	D-003229-07	CAACACAAGTACCTAATCA	1038
CDC27	NM_001256	16554576	996	D-003229-08	GGAGATGGATCCTATTTAC	1039
CDC27	NM_001256	16554576	996	D-003229-09	GAAAAGCCATGATGATATT	1040
CDC34						
CDC34	NM_004359	16357476	997	D-003230-05	GCTCAGACCTCTTCTACGA	1041
CDC34	NM_004359	16357476	997	D-003230-06	GGACGAGGGCGATCTATAC	1042
CDC34	NM_004359	16357476	997	D-003230-07	GATCGGGAGTACACAGACA	1043
CDC34	NM_004359	16357476	997	D-003230-08	TGAACGAGCCCAACACCTT	1044
CDC37						
CDC37	NM_007065	16357478	11140	D-003231-05	GCGAGGAGACAGCCAATTA	1045
CDC37	NM_007065	16357478	11140	D-003231-06	CACAAGACCTTCGTGGAAA	1046
CDC37	NM_007065	16357478	11140	D-003231-07	ACAATCGTCATGCAATTTA	1047
CDC37	NM_007065	16357478	11140	D-003231-08	GAGGAGAAATGTGCACTCA	1048
CDC45L						
CDC45L	NM_003504	34335230	8318	D-003232-05	GCACACGGATCTCCTTTGA	1049
CDC45L	NM_003504	34335230	8318	D-003232-06	GCAAACACCTGCTCAAGTC	1050
CDC45L	NM_003504	34335230	8318	D-003232-07	TGAAGAGTCTGCAAATAAA	1051
CDC45L	NM_003504	34335230	8318	D-003232-08	GGACGTGGATGCTCTGTGT	1052
CDC6						
CDC6	NM_001254	16357469	990	D-003233-05	GAACACAGCTGTCCCAGAT	1053
CDC6	NM_001254	16357469	990	D-003233-06	GAGCAGAGATGTCCACTGA	1054
CDC6	NM_001254	16357469	990	D-003233-07	GGAAATATCTTAGCTACTG	1055
CDC6	NM_001254	16357469	990	D-003233-08	GGACGAAGATTGGTATTTG	1056
CDC7						
CDC7	NM_003503	11038647	8317	D-003234-05	GGAATGAGGTACCTGATGA	1057
CDC7	NM_003503	11038647	8317	D-003234-06	CAGGAAAGGTGTTCACAAA	1058
CDC7	NM_003503	11038647	8317	D-003234-07	CTACACAAATGCACAAATT	1059
CDC7	NM_003503	11038647	8317	D-003234-08	GTACGGGAATATATGCTTA	1060
CDK10						
CDK10	NM_003674	32528262	8558	D-003235-05	GAACTGCTGTTGGGAACCA	1061
CDK10	NM_003674	32528262	8558	D-003235-06	GGAAGCAGCCCTACAACAA	1062
CDK10	NM_003674	32528262	8558	D-003235-07	GCACGCCCAGTGAGAACAT	1063
CDK10	NM_003674	32528262	8558	D-003235-08	GGAAGCAGCCCTACAACAA	1064
CDK2						
CDK2	NM_001798	16936527	1017	D-003236-05	GAGCTTAACCATCCTAATA	1065
CDK2	NM_001798	16936527	1017	D-003236-06	GAGCTTAACCATCCTAATA	1066
CDK2	NM_001798	16936527	1017	D-003236-07	GTACCGAGCTCCTGAAATC	1067

CDK2	NM_001798	16936527	1017	D-003236-08	GAGAGGTGGTGGCGCTTAA	1068
CDK3						
CDK3	NM_001258	4557438	1018	D-003237-05	GAGCATTGGTTGCATCTTT	1069
CDK3	NM_001258	4557438	1018	D-003237-06	GATCGGAGAGGGCACCTAT	1070
CDK3	NM_001258	4557438	1018	D-003237-07	GAAGCTCTATCTGGTGTTT	1071
CDK3	NM_001258	4557438	1018	D-003237-08	GCAGAGATGGTGACTCGAA	1072
CDK4						
CDK4	NM_000075	456426	1019	D-003238-05	GCAGCACTCTTATCTACAT	1073
CDK4	NM_000075	456426	1019	D-003238-06	GGAGGAGGCCTTCCCATCA	1074
CDK4	NM_000075	456426	1019	D-003238-07	TCGAAAGCCTCTCTTCTGT	1075
CDK4	NM_000075	456426	1019	D-003238-08	GTACCGAGCTCCCGAAGTT	1076
CDK5						
CDK5	NM_004935	4826674	1020	D-003239-05	TGACCAAGCTGCCAGACTA	1077
CDK5	NM_004935	4826674	1020	D-003239-06	GAGCTGAAATTGGCTGATT	1078
CDK5	NM_004935	4826674	1020	D-003239-07	CAACATCCCTGGTGAACGT	1079
CDK5	NM_004935	4826674	1020	D-003239-08	GGATTCCCGTCCGCTGTTA	1080
CDK6						
CDK6	NM_001259	16950658	1021	D-003240-05	GCAAAGACCTACTTCTGAA	1081
CDK6	NM_001259	16950658	1021	D-003240-06	GAAGAAGACTGGCCTAGAG	1082
CDK6	NM_001259	16950658	1021	D-003240-07	GGTCTGGACTTTCTTCATT	1083
CDK6	NM_001259	16950658	1021	D-003240-08	TAACAGATATCGATGAACT	1084
CDK7						
CDK7	NM_001799	16950659	1022	D-003241-05	GGACATAGATCAGAAGCTA	1085
CDK7	NM_001799	16950659	1022	D-003241-06	CAATAGAGCTTATACACAT	1086
CDK7	NM_001799	16950659	1022	D-003241-07	CATACAAGGCTTATTCTTA	1087
CDK7	NM_001799	16950659	1022	D-003241-08	GGAGACGACTTACTAGATC	1088
CDK8						
CDK8	NM_001260	4502744	1024	D-003242-05	CCACAGTACTCACATCAGA	1089
CDK8	NM_001260	4502744	1024	D-003242-06	GCAATAACCACACTAATGG	1090
CDK8	NM_001260	4502744	1024	D-003242-07	GAAGAAAGTGAGAGTTGTT	1091
CDK8	NM_001260	4502744	1024	D-003242-08	GAACATGACCTCTGGCATA	1092
CDK9						
CDK9	NM_001261	17017983	1025	D-003243-05	GGCCAAACGTGGACAACTA	1093
CDK9	NM_001261	17017983	1025	D-003243-06	TGACGTCCATGTTCGAGTA	1094
CDK9	NM_001261	17017983	1025	D-003243-07	CCAACCAGACGGAGTTTGA	1095
CDK9	NM_001261	17017983	1025	D-003243-08	GAAGGTGGCTCTGAAGAAG	1096
CDKN1C						
CDKN1C	NM_000076	4557440	1028	D-003244-05	GACCAGAACCGCTGGGATT	1097
CDKN1C	NM_000076	4557440	1028	D-003244-06	GGACCGAAGTGGACAGCGA	1098
CDKN1C	NM_000076	4557440	1028	D-003244-08	GCAAGAGATCAGCGCCTGA	1099
CDKN1C	NM_000076	4557440	1028	D-003244-09	CCGCTGGGATTACGACTTC	1100
CDKN2B						
CDKN2B	NM_004936	17981693	1030	D-003245-05	GCGAGGAGAACAAGGGCAT	1101
CDKN2B	NM_004936	17981693	1030	D-003245-06	CCAACGGAGTCAACCGTTT	1102
CDKN2B	NM_004936	17981693	1030	D-003245-07	CGATCCAGGTCATGATGAT	1103
CDKN2B	NM_004936	17981693	1030	D-003245-08	CCTGGAAGCCGGCGCGGAT	1104
CDKN2C						
CDKN2C	NM_001262	17981697	1031	D-003246-05	GGACACCGCCTGTGATTTG	1105
CDKN2C	NM_001262	17981697	1031	D-003246-06	GCCAGGAGACTGCTACTTA	1106
CDKN2C	NM_001262	17981697	1031	D-003246-07	TGAAAGACCGAACTGGTTT	1107
CDKN2C	NM_001262	17981697	1031	D-003246-08	GAACCTGCCCTTGCACTTG	1108
CDKN2D						
CDKN2D	NM_001800	17981700	1032	D-003247-05	TGGCAGTTCAAGAGGGTCA	1109
CDKN2D	NM_001800	17981700	1032	D-003247-06	CTCAGGACCTCGTGGACAT	1110
CDKN2D	NM_001800	17981700	1032	D-003247-07	TGAAGGTCCTAGTGGAGCA	1111
CDKN2D	NM_001800	17981700	1032	D-003247-08	AGACGGCGCTGCAGGTCAT	1112

CDT1						
CDT1	NM_030928	19923847	81620	D-003248-05	CCAAGGAGGCACAGAAGCA	1113
CDT1	NM_030928	19923847	81620	D-003248-06	GCTTCAACGTGGATGAAGT	1114
CDT1	NM_030928	19923847	81620	D-003248-07	TCTCCGGGCCAGAAGATAA	1115
CDT1	NM_030928	19923847	81620	D-003248-08	GCGCAATGTTGGCCAGATC	1116
CENPA						
CENPA	NM_001809	4585861	1058	D-003249-05	GCACACACCTCTTGATAAG	1117
CENPA	NM_001809	4585861	1058	D-003249-06	GCAAGAGAAATATGTGTTA	1118
CENPA	NM_001809	4585861	1058	D-003249-07	TTACATGCAGGCCGAGTTA	1119
CENPA	NM_001809	4585861	1058	D-003249-08	GAGACAAGGTTGGCTAAAG	1120
CENPB						
CENPB	NM_001810	26105977	1059	D-003250-05	GGACATAGCCGCCTGCTTT	1121
CENPB	NM_001810	26105977	1059	D-003250-06	GCACGATCCTGAAGAACAA	1122
CENPB	NM_001810	26105977	1059	D-003250-07	GGAGGAGGGTGATGTTGAT	1123
CENPB	NM_001810	26105977	1059	D-003250-08	CCGAATGGCTGCAGAGTCT	1124
CENPC1						
CENPC1	NM_001812	4502778	1060	D-003251-05	GCGAATAGATTATCAAGGA	1125
CENPC1	NM_001812	4502778	1060	D-003251-06	GAACAGAATCCATCACAAA	1126
CENPC1	NM_001812	4502778	1060	D-003251-07	CCATAAACCTCACCCAGTA	1127
CENPC1	NM_001812	4502778	1060	D-003251-08	CAAGAGAACACGTTTGAAA	1128
CENPE						
CENPE	NM_001813	4502780	1062	D-003252-05	GAAGACAGCTCAAATAATA	1129
CENPE	NM_001813	4502780	1062	D-003252-06	CAACAAAGCTACTAAATCA	1130
CENPE	NM_001813	4502780	1062	D-003252-07	GGAAAGAAGTGCTACCATA	1131
CENPE	NM_001813	4502780	1062	D-003252-08	GGAAAGAAATGACACAGTT	1132
CENPF						
CENPF	NM_016343	14670380	1063	D-003253-05	GCGAATATCTGAATTAGAA	1133
CENPF	NM_016343	14670380	1063	D-003253-06	GGAAATTAATGCATCCTTA	1134
CENPF	NM_016343	14670380	1063	D-003253-07	GAGCGAGGCTGGTGGTTTA	1135
CENPF	NM_016343	14670380	1063	D-003253-08	CAAGTCATCTTTCATCTAA	1136
CENPH						
CENPH	NM_022909	21264590	64946	D-003254-05	GAAAGAAGAGATTGCAATT	1137
CENPH	NM_022909	21264590	64946	D-003254-06	CAGAACAAATTATGCAAGA	1138
CENPH	NM_022909	21264590	64946	D-003254-07	CTAGTGTGCTCATGGATAA	1139
CENPH	NM_022909	21264590	64946	D-003254-08	GAAACACCTATTAGAGCTA	1140
CHEK1						
CHEK1	NM_001274	20127419	1111	D-003255-05	CAAATTGGATGCAGACAAA	1141
CHEK1	NM_001274	20127419	1111	D-003255-06	GCAACAGTATTTCGGTATA	1142
CHEK1	NM_001274	20127419	1111	D-003255-07	GGACTTCTCTCCAGTAAAC	1143
CHEK1	NM_001274	20127419	1111	D-003255-08	AAAGATAGATGGTACAACA	1144
CHEK2						
CHEK2	NM_007194	22209010	11200	D-003256-02	CTCTTACATTGCATACATA	1145
CHEK2	NM_007194	22209010	11200	D-003256-03	TAAACGCCTGAAAGAAGCT	1146
CHEK2	NM_007194	22209010	11200	D-003256-04	GCATAGGACTCAAGTGTCA	1147
CHEK2	NM_007194	22209010	11200	D-003256-05	GAAATTGCACTGTCACTAA	1148
CNK						
CNK	NM_004073	4758015	1263	D-003257-05	GCGAGAAGATCCTAAATGA	1149
CNK	NM_004073	4758015	1263	D-003257-07	GCAAGTGGGTTGACTACTC	1150
CNK	NM_004073	4758015	1263	D-003257-08	GCACATCCGTTGGCCATCA	1151
CNK	NM_004073	4758015	1263	D-003257-09	GACCTCAAGTTGGGAAATT	1152
CRI1						
CRI1	NM_014335	7656937	23741	D-003258-05	GTGATGAGATTATTGATAG	1153
CRI1	NM_014335	7656937	23741	D-003258-06	GGACGAGGGCGAGGAATTT	1154
CRI1	NM_014335	7656937	23741	D-003258-07	GGAAACGGAGCCTTGCTAA	1155
CRI1	NM_014335	7656937	23741	D-003258-08	TCAATCGTCTGACCGAAGA	1156
E2F1						

E2F1	NM_005225	12669910	1869	D-003259-05	GAACAGGGCCACTGACTCT	1157
E2F1	NM_005225	12669910	1869	D-003259-06	TGGACCACCTGATGAATAT	1158
E2F1	NM_005225	12669910	1869	D-003259-07	CCCAGGAGGTCACTTCTGA	1159
E2F1	NM_005225	12669910	1869	D-003259-08	GGCTGGACCTGGAAACTGA	1160
E2F2						
E2F2	NM_004091	34485718	1870	D-003260-05	GGGAGAAGACTCGGTATGA	1161
E2F2	NM_004091	34485718	1870	D-003260-06	GAGGACAACCTGCAGATAT	1162
E2F2	NM_004091	34485718	1870	D-003260-07	TGAAGGAGCTGATGAACAC	1163
E2F2	NM_004091	34485718	1870	D-003260-08	CCAAGAAGTTCATTTACCT	1164
E2F3						
E2F3	NM_001949	12669913	1871	D-003261-05	GAAATTAGATGAACTGATC	1165
E2F3	NM_001949	12669913	1871	D-003261-06	TGAAGTGCCTGACTCAATA	1166
E2F3	NM_001949	12669913	1871	D-003261-07	GAACAAGGCAGCAGAAGTG	1167
E2F3	NM_001949	12669913	1871	D-003261-08	GAAACACACAGTCCAATGA	1168
E2F4						
E2F4	NM_001950	12669914	1874	D-003262-05	GGAGATTGCTGACAAACTG	1169
E2F4	NM_001950	12669914	1874	D-003262-06	GAAGGTATCGGGCTAATCG	1170
E2F4	NM_001950	12669914	1874	D-003262-07	GTGCAGAAGTCCAGGGAAT	1171
E2F4	NM_001950	12669914	1874	D-003262-08	GGACAGTGGTGAGCTCAGT	1172
E2F5						
E2F5	NM_001951	12669916	1875	D-003263-05	GCAGATGACTACAACTTTA	1173
E2F5	NM_001951	12669916	1875	D-003263-06	GACATCAGCTACAGATATA	1174
E2F5	NM_001951	12669916	1875	D-003263-07	CAACATGTCTCTGAAAGAA	1175
E2F5	NM_001951	12669916	1875	D-003263-08	GAAGACATCTGTAATTGCT	1176
E2F6						
E2F6	NM_001952	12669917	1876	D-003264-05	TAAACAAGGTTGCAACGAA	1177
E2F6	NM_001952	12669917	1876	D-003264-06	TAGCATATGTGACCTATCA	1178
E2F6	NM_001952	12669917	1876	D-003264-07	GAAACCAGATTGGATGTTC	1179
E2F6	NM_001952	12669917	1876	D-003264-09	GGAACTTTCTGACTTATCA	1180
FOS						
FOS	NM_005252	6552332	2353	D-003265-05	GGGATAGCCTCTCTTACTA	1181
FOS	NM_005252	6552332	2353	D-003265-06	GAACAGTTATCTCCAGAAG	1182
FOS	NM_005252	6552332	2353	D-003265-07	GGAGACAGACCAACTAGAA	1183
FOS	NM_005252	6552332	2353	D-003265-08	AGACCGAGCCCTTTGATGA	1184
HIPK2						
HIPK2	NM_022740	13430859	28996	D-003266-06	GAGAATCACTCCAATCGAA	1185
HIPK2	NM_022740	13430859	28996	D-003266-07	AGACAGGGATTAAGTCAAA	1186
HIPK2	NM_022740	13430859	28996	D-003266-08	GGACAAAGACAACTAGGTT	1187
HIPK2	NM_022740	13430859	28996	D-003266-09	GCACACACGTCAAATCATG	1188
HUS1						
HUS1	NM_004507	31077213	3364	D-003267-05	ACAAAGGCCTTATGCAATA	1189
HUS1	NM_004507	31077213	3364	D-003267-06	GAAGTGCACATAGATATTA	1190
HUS1	NM_004507	31077213	3364	D-003267-07	AAGCTTAACTTCATCCTTT	1191
HUS1	NM_004507	31077213	3364	D-003267-08	GAACTTCTTCAACGAATTT	1192
JUN						
JUN	NM_002228	7710122	3725	D-003268-05	TGGAAACGACCTTCTATGA	1193
JUN	NM_002228	7710122	3725	D-003268-06	GAACTGCACAGCCAGAACA	1194
JUN	NM_002228	7710122	3725	D-003268-07	GAGCTGGAGCGCCTGATAA	1195
JUN	NM_002228	7710122	3725	D-003268-08	TAACGCAGCAGTTGCAAAC	1196
JUNB						
JUNB	NM_002229	4504808	3726	D-003269-05	GCATCAAAGTGGAGCGCAA	1197
JUNB	NM_002229	4504808	3726	D-003269-06	TGGAAGACCAAGAGCGCAT	1198
JUNB	NM_002229	4504808	3726	D-003269-07	CATACACAGCTACGGGATA	1199
JUNB	NM_002229	4504808	3726	D-003269-08	CCATCAACATGGAAGACCA	1200
LOC510 53						

LOC51053	NM_015895	20127542	51053	D-003270-05	GGAGAAAGGCGCTGTATGA	1201
LOC51053	NM_015895	20127542	51053	D-003270-06	GAATAGTTCTGTCCCAAGA	1202
LOC51053	NM_015895	20127542	51053	D-003270-07	GAACATGTACAGTATATGG	1203
LOC51053	NM_015895	20127542	51053	D-003270-08	GCAGAAACAAGAAGAAATC	1204
MAD2L1						
MAD2L1	NM_002358	6466452	4085	D-003271-05	GAAAGATGGCAGTTTGATA	1205
MAD2L1	NM_002358	6466452	4085	D-003271-06	TAAATAATGTGGTGGAACA	1206
MAD2L1	NM_002358	6466452	4085	D-003271-07	GAAATCCGTTCAGTGATCA	1207
MAD2L1	NM_002358	6466452	4085	D-003271-08	TTACTCGAGTGCAGAAATA	1208
MAD2L2						
MAD2L2	NM_006341	6006019	10459	D-003272-05	GGAAGAGCGCGCTCATAAA	1209
MAD2L2	NM_006341	6006019	10459	D-003272-06	TGGAAGAGCGCGCTCATAA	1210
MAD2L2	NM_006341	6006019	10459	D-003272-07	AGCCACTCCTGGAGAAGAA	1211
MAD2L2	NM_006341	6006019	10459	D-003272-08	TGGAGAAATTCGTCTTTGA	1212
MCM2						
MCM2	NM_004526	33356546	4171	D-003273-05	GAAGATCTTTGCCAGCATT	1213
MCM2	NM_004526	33356546	4171	D-003273-06	GGATAAGGCTCGTCAGATC	1214
MCM2	NM_004526	33356546	4171	D-003273-07	CAGAGCAGGTGACATATCA	1215
MCM2	NM_004526	33356546	4171	D-003273-08	GCCGTGGGCTCCTGTATGA	1216
MCM3						
MCM3	NM_002388	33356548	4172	D-003274-05	GGACATCAATATTCTTCTA	1217
MCM3	NM_002388	33356548	4172	D-003274-06	GCCAGGACATCTCCAGTTA	1218
MCM3	NM_002388	33356548	4172	D-003274-07	GCAGGTATGACCAGTATAA	1219
MCM3	NM_002388	33356548	4172	D-003274-08	GGAAATGCCTCAAGTACAC	1220
MCM4						
MCM4	XM_030274	22047061	4173	D-003275-05	GGACATATCTATTCTTACT	1221
MCM4	XM_030274	22047061	4173	D-003275-06	GATGTTAGTTCACCACTGA	1222
MCM4	XM_030274	22047061	4173	D-003275-07	CCAGCTGCCTCATACTTTA	1223
MCM4	XM_030274	22047061	4173	D-003275-08	GAAAGTACAAGATCGGTAT	1224
MCM5						
MCM5	NM_006739	23510447	4174	D-003276-05	GAAGATCCCTGGCATCATC	1225
MCM5	NM_006739	23510447	4174	D-003276-06	GAACAGGGTTACCATCATG	1226
MCM5	NM_006739	23510447	4174	D-003276-07	GGACAACATTGACTTCATG	1227
MCM5	NM_006739	23510447	4174	D-003276-08	CCAAGGAGGTAGCTGATGA	1228
MCM6						
MCM6	NM_005915	33469920	4175	D-003277-05	GGAAAGAGCTCAGAGATGA	1229
MCM6	NM_005915	33469920	4175	D-003277-06	GAGCAGCGATGGAGAAATT	1230
MCM6	NM_005915	33469920	4175	D-003277-07	GGAAACACCTGATGTCAAT	1231
MCM6	NM_005915	33469920	4175	D-003277-08	CCAAACATCTGCCGAAATC	1232
MCM7						
MCM7	NM_005916	33469967	4176	D-003278-05	GGAAATATCCCTCGTAGTA	1233
MCM7	NM_005916	33469967	4176	D-003278-06	GGAAGAAGCAGTTCAAGTA	1234
MCM7	NM_005916	33469967	4176	D-003278-07	CAACAAGCCTCGTGTGATC	1235
MCM7	NM_005916	33469967	4176	D-003278-08	GGAGAGAACACAAGGATTG	1236
MDM2						
MDM2	NM_002392	4505136	4193	D-003279-05	GGAGATATGTTGTGAAAGA	1237
MDM2	NM_002392	4505136	4193	D-003279-06	CCACAAATCTGATAGTATT	1238
MDM2	NM_002392	4505136	4193	D-003279-07	GATGAGGTATATCAAGTTA	1239
MDM2	NM_002392	4505136	4193	D-003279-08	GGAAGAAACCCAAGACAAA	1240
MKI67						
MKI67	NM_002417	19923216	4288	D-003280-05	GCACAAAGCTTGGTTATAA	1241
MKI67	NM_002417	19923216	4288	D-003280-06	CCTAAGACCTGAACTATTT	1242
MKI67	NM_002417	19923216	4288	D-003280-07	CAAAGAGGAACACAAATTA	1243

MKI67	NM_002417	19923216	4288	D-003280-08	GTAAATGGGTCTGTTATTG	1244
MNAT1						
MNAT1	NM_002431	4505224	4331	D-003281-05	GGAAGAAGCTTTAGAAGTG	1245
MNAT1	NM_002431	4505224	4331	D-003281-06	TAGATGAGCTGGAGAGTTC	1246
MNAT1	NM_002431	4505224	4331	D-003281-07	GGACCTTGCTGGAGGCTAT	1247
MNAT1	NM_002431	4505224	4331	D-003281-08	GCAGATAGAGACATATGGA	1248
MYC						
MYC	NM_002467	31543215	4609	D-003282-05	CAGAGAAGCTGGCCTCCTA	1249
MYC	NM_002467	31543215	4609	D-003282-06	GAAACGACGAGAACAGTTG	1250
MYC	NM_002467	31543215	4609	D-003282-07	CGACGAGACCTTCATCAAA	1251
MYC	NM_002467	31543215	4609	D-003282-08	CCACACATCAGCACAACTA	1252
ORC1L						
ORC1L	NM_004153	31795543	4998	D-003283-05	GAACAGGAATTCCAAGACA	1253
ORC1L	NM_004153	31795543	4998	D-003283-06	TAAGAAACGTGCTCGAGTA	1254
ORC1L	NM_004153	31795543	4998	D-003283-07	GAGATCACCTCACCTTCTA	1255
ORC1L	NM_004153	31795543	4998	D-003283-08	GCAGAGAGCCCTTCTTGGA	1256
ORC2L						
ORC2L	NM_006190	32454751	4999	D-003284-05	GAAGAAACCTCCTATGAGA	1257
ORC2L	NM_006190	32454751	4999	D-003284-06	GAAGGGAACTGATGGAGTA	1258
ORC2L	NM_006190	32454751	4999	D-003284-07	GAAGAATGATCCTGAGATT	1259
ORC2L	NM_006190	32454751	4999	D-003284-08	GAAGAGATGTTCAAGAATC	1260
ORC3L						
ORC3L	NM_012381	32483366	23595	D-003285-05	GGACTGCTGTGTAGATATA	1261
ORC3L	NM_012381	32483366	23595	D-003285-06	GAACTGATGACCATACTTG	1262
ORC3L	NM_012381	32483366	23595	D-003285-07	AAAGATCTCTCTGCCAATA	1263
ORC3L	NM_012381	32483366	23595	D-003285-08	CAGCACAGCTAAGAGAATA	1264
ORC4L						
ORC4L	NM_002552	32454749	5000	D-003286-06	GAAAGCACATTCCGTTTAT	1265
ORC4L	NM_002552	32454749	5000	D-003286-07	TGAAAGAACTCATGGAAAT	1266
ORC4L	NM_002552	32454749	5000	D-003286-08	GCTGAGAAGTGGAATGAAA	1267
ORC4L	NM_002552	32454749	5000	D-003286-09	CCAGTGATCTTCATATTAG	1268
ORC5L						
ORC5L	NM_002553	32454752	5001	D-003287-05	GAAATAACCTGTGAAACAT	1269
ORC5L	NM_002553	32454752	5001	D-003287-06	CAGATTACCTCTCTAGTGA	1270
ORC5L	NM_002553	32454752	5001	D-003287-07	GAACTTCCATATTACTCTA	1271
ORC5L	NM_002553	32454752	5001	D-003287-08	GTATTCAGCTGATTTCTAT	1272
ORC6L						
ORC6L	NM_014321	32454755	23594	D-003288-05	GAACATGGCTTCAAAGATA	1273
ORC6L	NM_014321	32454755	23594	D-003288-06	GGACAGGGCTTATTTAATT	1274
ORC6L	NM_014321	32454755	23594	D-003288-07	GAAAGAAGATAGTGGTTGA	1275
ORC6L	NM_014321	32454755	23594	D-003288-08	TATCAGAGCTGTCTTAAAT	1276
PCNA						
PCNA	NM_002592	33239449	5111	D-003289-05	GATCGAGGATGAAGAAGGA	1277
PCNA	NM_002592	33239449	5111	D-003289-07	GCCGAGATCTCAGCCATAT	1278
PCNA	NM_002592	33239449	5111	D-003289-09	GAGGCCTGCTGGGATATTA	1279
PCNA	NM_002592	33239449	5111	D-003289-10	GTGGAGAACTTGGAAATGG	1280
PLK						
PLK	NM_005030	21359872	5347	D-003290-05	CAACCAAAGTCGAATATGA	1281
PLK	NM_005030	21359872	5347	D-003290-06	CAAGAAGAATGAATACAGT	1282
PLK	NM_005030	21359872	5347	D-003290-07	GAAGATGTCCATGGAAATA	1283
PLK	NM_005030	21359872	5347	D-003290-08	CAACACGCCTCATCCTCTA	1284
PIN1						
PIN1	NM_006221	5453897	5300	D-003291-05	GGACCAAGGAGGAGGCCCT	1285
PIN1	NM_006221	5453897	5300	D-003291-06	CGTCCTGGCGGCAGGAGAA	1286
PIN1	NM_006221	5453897	5300	D-003291-07	CGGGAGAGGAGGACTTTGA	1287
PIN1	NM_006221	5453897	5300	D-003291-08	AGTCGGGAGAGGAGGACTT	1288

PIN1L						
PIN1L	NM_006222	5453899	5301	D-003292-06	CGACCTGGCGGCAGGAAAT	1289
PIN1L	NM_006222	5453899	5301	D-003292-07	AGGCAGGAGAGAAGGACTT	1290
PIN1L	NM_006222	5453899	5301	D-003292-08	GCTACATCCAGAAGATCAA	1291
PIN1L	NM_006222	5453899	5301	D-003292-09	GGACAGTGTTCACGGATTC	1292
RAD1						
RAD1	NM_002853	19718797	5810	D-003293-05	GAAGATGGACAAATATGTT	1293
RAD1	NM_002853	19718797	5810	D-003293-06	GGAAGAGTCTGTTACTTTT	1294
RAD1	NM_002853	19718797	5810	D-003293-07	GATAACAGAGGCTTCCTTT	1295
RAD1	NM_002853	19718797	5810	D-003293-08	GCATTAGTCCTATCTTGTA	1296
RAD17						
RAD17	NM_133338	19718783	5884	D-003294-05	GAATCAAGCTTCCATATGT	1297
RAD17	NM_133338	19718783	5884	D-003294-06	CAACAAAGCCCGAGGATAT	1298
RAD17	NM_133338	19718783	5884	D-003294-07	ACACATGCCTGGAGACTTA	1299
RAD17	NM_133338	19718783	5884	D-003294-08	CTACATAGATTTCTTCATG	1300
RAD9A						
RAD9A	NM_004584	19924112	5883	D-003295-05	TCAGCAAACTTGAATCTTA	1301
RAD9A	NM_004584	19924112	5883	D-003295-06	GACATTGACTCTTACATGA	1302
RAD9A	NM_004584	19924112	5883	D-003295-08	GGAAACCACTATAGGCAAT	1303
RAD9A	NM_004584	19924112	5883	D-003295-09	CGGACGACTTTGCCAATGA	1304
RB1						
RB1	NM_000321	19924112	5925	D-003296-05	GAAAGGACATGTGAACTTA	1305
RB1	NM_000321	19924112	5925	D-003296-06	GAAGAAGTATGATGTATTG	1306
RB1	NM_000321	4506434	5925	D-003296-07	GAAATGACTTCTACTCGAA	1307
RB1	NM_000321	4506434	5925	D-003296-08	GGAGGGAACATCTATATTT	1308
RBBP2						
RBBP2	NM_005056	4826967	5927	D-003297-05	CAAAGAAGCTGAATAAACT	1309
RBBP2	NM_005056	4826967	5927	D-003297-06	CAACACATATGGCGGATTT	1310
RBBP2	NM_005056	4826967	5927	D-003297-07	GGACAAACCTAGAAAGAAG	1311
RBBP2	NM_005056	4826967	5927	D-003297-08	GAAAGGCACTCTCTCTGTT	1312
RBL1						
RBL1	NM_002895	34577078	5933	D-003298-05	CAAGAGAAGTTGTGGCATA	1313
RBL1	NM_002895	34577078	5933	D-003298-06	CAGCAGCACTCCATTTATA	1314
RBL1	NM_002895	34577078	5933	D-003298-07	ACAGAAAGGTCTATCATTT	1315
RBL1	NM_002895	34577078	5933	D-003298-08	GGACATAAAGTTACAATTC	1316
RBL2						
RBL2	NM_005611	21361291	5934	D-003299-05	GAGCAGAGCTTAATCGAAT	1317
RBL2	NM_005611	21361291	5934	D-003299-06	GAGAATAGCCCTTGTGTGA	1318
RBL2	NM_005611	21361291	5934	D-003299-07	GGACTTAGTTTATGGAAAT	1319
RBL2	NM_005611	21361291	5934	D-003299-08	GAATTTAGATGAGCGGATA	1320
RBP1						
RBP1	NM_002899	8400726	5947	D-003300-05	GAGACAAGCTCCAGTGTGT	1321
RBP1	NM_002899	8400726	5947	D-003300-06	GCAAGCAAGTATTCAAGAA	1322
RBP1	NM_002899	8400726	5947	D-003300-07	GCAGGACGGTGACCATATG	1323
RBP1	NM_002899	8400726	5947	D-003300-08	GCAAGTGCATGACAACAGT	1324
RPA3						
RPA3	NM_002947	19923751	6119	D-003322-05	GGAAGTGGTTGGAAGAGTA	1325
RPA3	NM_002947	19923751	6119	D-003322-06	GAAGATAGCCATCCTTTTG	1326
RPA3	NM_002947	19923751	6119	D-003322-07	CATGCTAGCTCAATTCATC	1327
RPA3	NM_002947	19923751	6119	D-003322-08	GATCTTGGACTTTACAATG	1328
SKP1A						
SKP1A	NM_006930	25777710	6500	D-003323-05	GGAGAGATATTTGAAGTTG	1329
SKP1A	NM_006930	25777710	6500	D-003323-06	GGGAATGGATGATGAAGGA	1330
SKP1A	NM_006930	25777710	6500	D-003323-07	CAAACAATCTGTGACTATT	1331
SKP1A	NM_006930	25777710	6500	D-003323-08	TCAATTAAGTTGCAGAGTT	1332
SKP2						

SKP2	NM_005983	16306594	6502	D-003324-05	CATCTAGACTTAAGTGATA	1333
SKP2	NM_005983	16306594	6502	D-003324-06	GAAATCAGATCTCTCTACT	1334
SKP2	NM_005983	16306594	6502	D-003324-07	CTAAAGGTCTCTGGTGTTT	1335
SKP2	NM_005983	16306594	6502	D-003324-08	GATGGTACCCTTCAACTGT	1336
SNK						
SNK	NM_006622	5730054	10769	D-003325-05	GAAGACATCTACAAGCTTA	1337
SNK	NM_006622	5730054	10769	D-003325-06	GAAATACCTTCATGAACAA	1338
SNK	NM_006622	5730054	10769	D-003325-07	GAAGGTCAATGGCTCATAT	1339
SNK	NM_006622	5730054	10769	D-003325-08	CCGGAGATCTCGCGGATTA	1340
STK12						
STK12	NM_004217	4759177	9212	D-003326-07	CAGAAGAGCTGCACATTTG	1341
STK12	NM_004217	4759177	9212	D-003326-08	CCAAACTGCTCAGGCATAA	1342
STK12	NM_004217	4759177	9212	D-003326-09	ACGCGGCACTTCACAATTG	1343
STK12	NM_004217	4759177	9212	D-003326-10	TGGGACACCCGACATCTTA	1344
TFDP1						
TFDP1	NM_007111	34147667	7027	D-003327-05	GGAAGCAGCTCTTGCCAAA	1345
TFDP1	NM_007111	34147667	7027	D-003327-06	GAGGAGACTTGAAAGAATA	1346
TFDP1	NM_007111	34147667	7027	D-003327-07	GAACTTAGAGGTGGAAAGA	1347
TFDP1	NM_007111	34147667	7027	D-003327-08	GCGAGAAGGTGCAGAGGAA	1348
TFDP2						
TFDP2	NM_006286	5454111	7029	D-003328-05	GAAAGTGTGTGAGAAAGTT	1349
TFDP2	NM_006286	5454111	7029	D-003328-06	CACAGGACCTTCTTGGTTA	1350
TFDP2	NM_006286	5454111	7029	D-003328-07	CGAAATCCCTGGTGCCAAA	1351
TFDP2	NM_006286	5454111	7029	D-003328-08	TGAGATCCATGATGACATA	1352
TP53						
TP53	NM_000546	8400737	7157	D-003329-05	GAGGTTGGCTCTGACTGTA	1353
TP53	NM_000546	8400737	7157	D-003329-06	CAGTCTACCTCCCGCCATA	1354
TP53	NM_000546	8400737	7157	D-003329-07	GCACAGAGGAAGAGAATCT	1355
TP53	NM_000546	8400737	7157	D-003329-08	GAAGAAACCACTGGATGGA	1356
TP63						
TP63	NM_003722	31543817	8626	D-003330-05	CATCATGTCTGGACTATTT	1357
TP63	NM_003722	31543817	8626	D-003330-06	CAAACAAGATTGAGATTAG	1358
TP63	NM_003722	31543817	8626	D-003330-07	GCACACAGACAAATGAATT	1359
TP63	NM_003722	31543817	8626	D-003330-08	CGACAGTCTTGTACAATTT	1360
TP73						
TP73	NM_005427	4885644	7161	D-003331-05	GCAAGCAGCCCATCAAGGA	1361
TP73	NM_005427	4885644	7161	D-003331-06	GAGACGAGGACACGTACTA	1362
TP73	NM_005427	4885644	7161	D-003331-07	CTGCAGAACCTGACCATTG	1363
TP73	NM_005427	4885644	7161	D-003331-08	GGCCATGCCTGTTTACAAG	1364
YWHAZ						
YWHAZ	NM_003406	21735623	7534	D-003332-05	GCAAGGAGCTGAATTATCC	1365
YWHAZ	NM_003406	21735623	7534	D-003332-06	TAAGAGATATCTGCAATGA	1366
YWHAZ	NM_003406	21735623	7534	D-003332-07	GACGGAAGGTGCTGAGAAA	1367
YWHAZ	NM_003406	21735623	7534	D-003332-08	AGAGCAAAGTCTTCTATTT	1368

Table IX

Gene Name	Accession #	GI#	Duplex #	Sequence	SEQ. ID NO.
AR	NM_000044	21322251	D-003400-01	GGAACTCGATCGTATCATT	1369
AR	NM_000044	21322251	D-003400-02	CAAGGGAGGTTACACCAAA	1370
AR	NM_000044	21322251	D-003400-03	TCAAGGAACTCGATCGTAT	1371
AR	NM_000044	21322251	D-003400-04	GAAATGATTGCACTATTGA	1372
ESR1	NM_000125	4503602	D-003401-01	GAATGTGCCTGGCTAGAGA	1373
ESR1	NM_000125	4503602	D-003401-02	CATGAGAGCTGCCAACCTT	1374
ESR1	NM_000125	4503602	D-003401-03	AGAGAAAGATTGGCCAGTA	1375

ESR1	NM_000125	4503602	D-003401-04	CAAGGAGACTCGCTACTGT	1376
ESR2	NM_001437	10835012	D-003402-01	GAACATCTGCTCAACATGA	1377
ESR2	NM_001437	10835012	D-003402-02	GCACGGCTCCATATACATA	1378
ESR2	NM_001437	10835012	D-003402-03	CAAGAAGATTCCCGGCTTT	1379
ESR2	NM_001437	10835012	D-003402-04	GGAAATGCGTAGAAGGAAT	1380
ESRRA	NM_004451	18860919	D-003403-01	GGCCTTCGCTGAGGACTTA	1381
ESRRA	NM_004451	18860919	D-003403-02	TGAATGCACTGGTGTCTCA	1382
ESRRA	NM_004451	18860919	D-003403-03	GCATTGAGCCTCTCTACAT	1383
ESRRA	NM_004451	18860919	D-003403-04	CCAGACAGCGGGCAAAGTG	1384
ESRRB	NM_004452	22035686	D-003404-01	TACCTGAGCTTACAAATTT	1385
ESRRB	NM_004452	22035686	D-003404-02	GCACTTCTATAGCGTCAAA	1386
ESRRB	NM_004452	22035686	D-003404-03	CAACTCCGATTCCATGTAC	1387
ESRRB	NM_004452	22035686	D-003404-04	GGACTCGCCACCCATGTTT	1388
ESRRG	NM_001438	4503604	D-003405-01	AAACAAAGATCGACACATT	1389
ESRRG	NM_001438	4503604	D-003405-02	TCAGGAAACTGTATGATGA	1390
ESRRG	NM_001438	4503604	D-003405-03	GAAGACCAGTCCAAATTAG	1391
ESRRG	NM_001438	4503604	D-003405-04	ATGAAGCGCTGCAGGATTA	1392
HNF4A	NM_000457	21361184	D-003406-01	CGACATCACTGGAGCATAT	1393
HNF4A	NM_000457	21361184	D-003406-02	GAAGGAAGCCGTCCAGAAT	1394
HNF4A	NM_000457	21361184	D-003406-03	CCAAGTACATCCCAGCTTT	1395
HNF4A	NM_000457	21361184	D-003406-04	GGACATGGCCGACTACAGT	1396
HNF4G	NM_004133	6631087	D-003407-01	GCACTGACATAAACGTTAA	1397
HNF4G	NM_004133	6631087	D-003407-02	ACAAAGAGATCCATGATGT	1398
HNF4G	NM_004133	6631087	D-003407-03	AGAGATCCATGATGTATAA	1399
HNF4G	NM_004133	6631087	D-003407-04	AAATGAACGTGACAGAATA	1400
HSAJ2425	NM_017532	8923776	D-003408-01	GAATGAATCTACACCTTTG	1401
HSAJ2425	NM_017532	8923776	D-003408-02	GGAAATACGTGGAGACACT	1402
HSAJ2425	NM_017532	8923776	D-003408-03	CCAGATAACTACGGCGATA	1403
HSAJ2425	NM_017532	8923776	D-003408-04	TGGCGTACCTTCTCATTGA	1404
NR0B1	NM_000475	5016089	D-003409-01	CAGCATGGATGATATGATG	1405
NR0B1	NM_000475	5016089	D-003409-02	CTGCTGAGATTCATCAATG	1406
NR0B1	NM_000475	5016089	D-003409-03	ACAGATTCATCGAACTTAA	1407
NR0B1	NM_000475	5016089	D-003409-04	GAACGTGGCGCTCCTGTAC	1408
NR0B2	NM_021969	13259502	D-003410-01	GAATATGCCTGCCTGAAAG	1409
NR0B2	NM_021969	13259502	D-003410-02	GGAATATGCCTGCCTGAAA	1410
NR0B2	NM_021969	13259502	D-003410-03	CGTAGCCGCTGCCTATGTA	1411
NR0B2	NM_021969	13259502	D-003410-04	GCCATTCTCTACGCACTTC	1412
NR1D1	NM_021724	13430847	D-003411-01	CAACACAGGTGGCGTCATCTT	1413
NR1D1	NM_021724	13430847	D-003411-02	GGCATGGTGTTACTGTGTATT	1414
NR1D1	NM_021724	13430847	D-003411-03	CAACATGCATTCCGAGAAGTT	1415
NR1D1	NM_021724	13430847	D-003411-04	GCGCTTTGCTTCGTTGTTCTT	1416
NR1H2	NM_007121	11321629	D-003412-01	GAACAGATCCGGAAGAAGA	1417
NR1H2	NM_007121	11321629	D-003412-02	GAAGAACAGATCCGGAAGA	1418
NR1H2	NM_007121	11321629	D-003412-03	CTAAGCAAGTGCCTGGTTT	1419
NR1H2	NM_007121	11321629	D-003412-04	GCTAACAGCGGCTCAAGAA	1420

NR1H3	NM_005693	5031892	D-003413-01	GAACAGATCCGCCTGAAGA	1421
NR1H3	NM_005693	5031892	D-003413-02	GGAGATAGTTGACTTTGCT	1422
NR1H3	NM_005693	5031892	D-003413-03	GAGTTTGCCTTGCTCATTG	1423
NR1H3	NM_005693	5031892	D-003413-04	TGACTTTGCTAAACAGCTA	1424
NR1H4	NM_005123	4826979	D-003414-01	CAAGTGACCTCGACAACAA	1425
NR1H4	NM_005123	4826979	D-003414-02	GAAAGAATTCGAAATAGTG	1426
NR1H4	NM_005123	4826979	D-003414-03	CAACAGACTCTTCTACATT	1427
NR1H4	NM_005123	4826979	D-003414-04	GAACCATACTCGCAATACA	1428
NR1I2	NM_003889	11863133	D-003415-01	GAACCATGCTGACTTTGTA	1429
NR1I2	NM_003889	11863133	D-003415-02	GATGGACGCTCAGATGAAA	1430
NR1I2	NM_003889	11863133	D-003415-03	CAACCTACATGTTCAAAGG	1431
NR1I2	NM_003889	11863133	D-003415-04	CAGGAGCAATTCGCCATTA	1432
NR1I3	NM_005122	4826660	D-003416-01	GGAAATCTGTCACATCGTA	1433
NR1I3	NM_005122	4826660	D-003416-02	TCGCAGACATCAACACTTT	1434
NR1I3	NM_005122	4826660	D-003416-03	CCTCTTCGCTACACAATTG	1435
NR1I3	NM_005122	4826660	D-003416-04	GAACAGTTTGTGCAGTTTA	1436
NR2C1	NM_003297	4507672	D-003417-01	TGACAGCACTTGATCATAA	1437
NR2C1	NM_003297	4507672	D-003417-02	GGAAGGAAGTGTACACCTA	1438
NR2C1	NM_003297	4507672	D-003417-03	GAGCACATCTTCAAACTAC	1439
NR2C1	NM_003297	4507672	D-003417-04	GAAGAAATTGCACATCAAA	1440
NR2C2	NM_003298	4507674	D-003418-01	GAACAACGGTGACACTTCA	1441
NR2C2	NM_003298	4507674	D-003418-02	CTGATGAGCTCCAACATAA	1442
NR2C2	NM_003298	4507674	D-003418-03	CAACCTAAGTGAATCTTTG	1443
NR2C2	NM_003298	4507674	D-003418-04	GAAGACACCTACCGATTGG	1444
NR2E1	NM_003269	21361108	D-003419-01	GATCATATCTGAAATACAG	1445
NR2E1	NM_003269	21361108	D-003419-02	CAAGACTGCTTTCAGATAT	1446
NR2E1	NM_003269	21361108	D-003419-03	GTTAGATGCTACTGAATTT	1447
NR2E1	NM_003269	21361108	D-003419-04	CAATGTATCTCTATGAAGT	1448
NR2E3	NM_014249	7657394	D-003420-01	GAGAAGCTCCTTTGTGATA	1449
NR2E3	NM_014249	7657394	D-003420-02	GAAGCACTATGGCATCTAT	1450
NR2E3	NM_014249	7657394	D-003420-03	GAAGGATCCTGAGCACGTA	1451
NR2E3	NM_014249	7657394	D-003420-04	GAAGCTCCTTTGTGATATG	1452
NR2F1	NM_005654	20127484	D-003421-01	GAAACTCTCATCCGCGATA	1453
NR2F1	NM_005654	20127484	D-003421-02	TCTCATCCGCGATATGTTA	1454
NR2F1	NM_005654	20127484	D-003421-03	CAAGAAGTGCCTCAAAGTG	1455
NR2F1	NM_005654	20127484	D-003421-04	GGAACTTAACTTACACATG	1456
NR2F2	NM_021005	14149745	D-003422-01	GTACCTGTCCGGATATATT	1457
NR2F2	NM_021005	14149745	D-003422-02	CCAACCAGCCGACGAGATT	1458
NR2F2	NM_021005	14149745	D-003422-03	ACTCGTACCTGTCCGGATA	1459
NR2F2	NM_021005	14149745	D-003422-04	GGCCGTATATGGCAATTCA	1460
NR2F6	NM_005234	20070198	D-003423-01	CGACGCCTGTGGCCTCTCA	1461
NR2F6	NM_005234	20070198	D-003423-02	CAGCCGGTGTCCGAACTGA	1462
NR2F6	NM_005234	20070198	D-003423-03	CAACCGTGACTGCCAGATC	1463
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NR3C1	NM_000176	4504132	D-003424-01	GAGGACAGATGTACCACTA	1465
NR3C1	NM_000176	4504132	D-003424-02	GATAAGACCATGAGTATTG	1466
NR3C1	NM_000176	4504132	D-003424-03	GAAGACGATTCATTCCTTT	1467
NR3C1	NM_000176	4504132	D-003424-04	GGACAGATGTACCACTATG	1468
NR3C2	NM_000901	4505198	D-003425-01	GCAAACAGATGATCCAAGT	1469
NR3C2	NM_000901	4505198	D-003425-02	CAGCTAAGATTTATCAGAA	1470
NR3C2	NM_000901	4505198	D-003425-03	GCACGAAAGTCAAAGAAGT	1471
NR3C2	NM_000901	4505198	D-003425-04	GGTATCCGGTCTTAGAATA	1472
NR4A1	NM_002135	21361341	D-003426-01	GAAGGAAGTTGTCCGAACA	1473
NR4A1	NM_002135	21361341	D-003426-02	CAGGAGAGTTTGACACCTT	1474
NR4A1	NM_002135	21361341	D-003426-03	CAGTGGCTCTGACTACTAT	1475
NR4A1	NM_002135	21361341	D-003426-04	GAAGGCCGCTGTGCTGTGT	1476
NR4A2	NM_006186	5453821	D-003427-01	GCAATGCGTTCGTGGCTTT	1477
NR4A2	NM_006186	5453821	D-003427-02	CGGCTACACAGGAGAGTTT	1478
NR4A2	NM_006186	5453821	D-003427-03	CCACGTGACTTTCAACAAT	1479
NR4A2	NM_006186	5453821	D-003427-04	GAATACAGCTCCGATTTCT	1480
NR4A3	NM_006981	11276070	D-003428-01	CAAAGAAGATCAGACATTA	1481
NR4A3	NM_006981	11276070	D-003428-02	GATCAGACATTACTTATTG	1482
NR4A3	NM_006981	11276070	D-003428-03	CCAGAGATCTTGATTATTC	1483
NR4A3	NM_006981	11276070	D-003428-04	GAAGTTGTCCGTACAGATA	1484
NR5A1	NM_004959	20070192	D-003429-01	GATTTGAAGTTCCTGAATA	1485
NR5A1	NM_004959	20070192	D-003429-02	GGAGCGAGCTGCTGGTGTT	1486
NR5A1	NM_004959	20070192	D-003429-03	GGAGGTGGCCGACCAGATG	1487
NR5A1	NM_004959	20070192	D-003429-04	CAACGTGCCTGAGCTCATC	1488
NR5A2	NM_003822	20070161	D-003430-01	CCAAACATATGGCCACTTT	1489
NR5A2	NM_003822	20070161	D-003430-02	TCAGAGAACTTAAGGTTGA	1490
NR5A2	NM_003822	20070161	D-003430-03	GGATCCATCTTCCTGGTTA	1491
NR5A2	NM_003822	20070161	D-003430-04	AAGAATACCTCTACTACAA	1492
NR6A1	NM_033334	15451847	D-003431-01	CAACGAACCTGTCTCATTT	1493
NR6A1	NM_033334	15451847	D-003431-02	GAAGAACTACACAGATTTA	1494
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NR6A1	NM_033334	15451847	D-003431-04	AAACGATACTGGTACATTT	1496
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null	D16815	2116671	D-003432-03	GAGGAGCTCTTGGCCTTTA	1499
null	D16815	2116671	D-003432-04	TAAACAACATGCACTCTGA	1500
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PGR	NM_000926	4505766	D-003433-02	CAGCGTTTCTATCAACTTA	1502
PGR	NM_000926	4505766	D-003433-03	AGATAACTCTCATTCAGTA	1503
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PPARA	NM_005036	7549810	D-003434-02	GAACATGACATAGAAGATT	1506
PPARA	NM_005036	7549810	D-003434-03	GGATAGTTCTGGAAGCTTT	1507
PPARA	NM_005036	7549810	D-003434-04	GACTCAAGCTGGTGTATGA	1508
PPARD	NM_006238	5453939	D-003435-01	GAGCGCAGCTGCAAGATTC	1509

PPARD	NM_006238	5453939	D-003435-02	GCATGAAGCTGGAGTACGA	1510
PPARD	NM_006238	5453939	D-003435-03	GGAAGCAGTTGGTGAATGG	1511
PPARD	NM_006238	5453939	D-003435-04	GCTGCAAGATTCAGAAGAA	1512
PPARG	NM_138712	20336234	D-003436-01	AGACTCAGCTCTACAATAA	1513
PPARG	NM_138712	20336234	D-003436-02	GATTGAAGCTTATCTATGA	1514
PPARG	NM_138712	20336234	D-003436-03	AAGTAACTCTCCTCAAATA	1515
PPARG	NM_138712	20336234	D-003436-04	GCATTTCTACTCCACATTA	1516
RARA	NM_000964	4506418	D-003437-01	GACAAGAACTGCATCATCA	1517
RARA	NM_000964	4506418	D-003437-02	GCAAATACACTACGAACAA	1518
RARA	NM_000964	4506418	D-003437-03	GAACAACAGCTCAGAACAA	1519
RARA	NM_000964	4506418	D-003437-04	GAGCAGCAGTTCTGAAGAG	1520
RARB	NM_000965	14916493	D-003438-01	GCACACTGCTCAATCAATT	1521
RARB	NM_000965	14916493	D-003438-02	GCAGAAGTATTCAGAAGAA	1522
RARB	NM_000965	14916493	D-003438-03	GGAATGACAGGAACAAGAA	1523
RARB	NM_000965	14916493	D-003438-04	GCACAGTCCTAGCATCTCA	1524
RARG	NM_000966	21359851	D-003439-01	GAAATGACCGGAACAAGAA	1525
RARG	NM_000966	21359851	D-003439-02	TAGAAGAGCTCATCACCAA	1526
RARG	NM_000966	21359851	D-003439-03	CAAGGAAGCTGTGCGAAAT	1527
RARG	NM_000966	21359851	D-003439-04	TCAGTGAGCTGGCTACCAA	1528
RORA	NM_134261	19743902	D-003440-01	GGAAAGAGTTTATGTTCTA	1529
RORA	NM_134261	19743902	D-003440-02	CAAGATCTGTGGAGACAAA	1530
RORA	NM_134261	19743902	D-003440-03	GCACCTGACTGAAGATGAA	1531
RORA	NM_134261	19743902	D-003440-04	CCGAGAAGATGGAATACTA	1532
RORB	NM_006914	19743906	D-003441-01	GCACAGAACATCATTAAGT	1533
RORB	NM_006914	19743906	D-003441-02	CCACACCTATGAAGAAATT	1534
RORB	NM_006914	19743906	D-003441-03	GATCAAATTCTACTTCTGA	1535
RORB	NM_006914	19743906	D-003441-04	TCAAACAGATAAAGCAAGA	1536
RORC	NM_005060	19743908	D-003442-01	TAGAACAGCTGCAGTACAA	1537
RORC	NM_005060	19743908	D-003442-02	TCACCGAGGCCATTCAGTA	1538
RORC	NM_005060	19743908	D-003442-03	GAACAGCTGCAGTACAATC	1539
RORC	NM_005060	19743908	D-003442-04	CCTCATGCCACCTTGAATA	1540
RXRA	NM_002957	21536318	D-003443-01	TGACGGAGCTTGTGTCCAA	1541
RXRA	NM_002957	21536318	D-003443-02	CAACAAGGACTGCCTGATT	1542
RXRA	NM_002957	21536318	D-003443-03	GCAAGGACCTGACCTACAC	1543
RXRA	NM_002957	21536318	D-003443-04	GCAAGGACCGGAACGAGAA	1544
RXRB	NM_021976	21687229	D-003444-01	GCAAAGACCTTACATACTC	1545
RXRB	NM_021976	21687229	D-003444-02	GCAATCATTCTGTTTAATC	1546
RXRB	NM_021976	21687229	D-003444-03	TCACACCGATCCATTGATG	1547
RXRB	NM_021976	21687229	D-003444-04	GCAAACGGCTATGTGCAAT	1548
RXRG	NM_006917	21361386	D-003445-01	GGAAGGACCTCATCTACAC	1549
RXRG	NM_006917	21361386	D-003445-02	CCGGATCTCTGGTTAAACA	1550
RXRG	NM_006917	21361386	D-003445-03	GCGAGCCATTGTACTCTTT	1551
RXRG	NM_006917	21361386	D-003445-04	GAGCCATTGTACTCTTTAA	1552
THRA	NM_003250	20127451	D-003446-01	GGACAAAGACGAGCAGTGT	1553
THRA	NM_003250	20127451	D-003446-02	GGAAACAGAGGCGGAAATT	1554

THRA	NM_003250	20127451	D-003446-03	GTAAGCTGATTGAGCAGAA	1555
THRA	NM_003250	20127451	D-003446-04	GAACCTCCATCCCACCTAT	1556
THRB	NM_000461	10835122	D-003447-01	GAATGTCGCTTTAAGAAAT	1557
THRB	NM_000461	10835122	D-003447-02	GAACAGTCGTCGCCACATC	1558
THRB	NM_000461	10835122	D-003447-03	GGACAAGCACCAATAGTCA	1559
THRB	NM_000461	10835122	D-003447-04	GTGGAAAGGTTGACTTGGA	1560
VDR	NM_000376	4507882	D-003448-01	TGAAGAAGCTGAACTTGCA	1561
VDR	NM_000376	4507882	D-003448-02	GCAACCAAGACTACAAGTA	1562
VDR	NM_000376	4507882	D-003448-03	TCAATGCTATGACCTGTGA	1563
VDR	NM_000376	4507882	D-003448-04	CCATTGAGGTCATCATGTT	1564

Table X

Gene Symbol	Sense	SEQ ID NO
ABCB1	GACCAUAAAUGUAAGGUUU	1565
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	GAAAUGUUCACUUCAGUUA	1567
	GAAGAUCGCUACUGAAGCA	1568
ABCC1	Sense	
	GGAAGCAACUGCAGAGACA	1569
	GAUGACACCUCUCAACAAA	1570
	UAAAGUUGCUCAUCAAGUU	1571
	CAACGAGUCUGCCGAAGGA	1572
ABCG2	Sense	
	GCAGAUGCCUUCUUCGUUA	1573
	AGGCAAAUCUUCGUUAUUA	1574
	GGGAAGAAAUCUGGUCUAA	1575
	UGACUCAUCCCAACAUUUA	1576
KCNH2	Sense	
	CCGACGUGCUGCCUGAGUA	1577
	GAGAAGAGCAGCGACACUU	1578
	GAUCAUAGCACCUAAGAUA	1579
	GCUAUUUACUGCUCUUAUU	1580
	UCACUGGGCUCCUUUAAUU	1581
	GUGCGAGCCUUCUGAAUAU	1582
	GCUAAGCUAUACUACUGUA	1583
	UGACGGCGCUCUACUUCAC	1584
KCNH1	Sense	
	GAGAUGAAUUCCUUUGAAA	1585
	GAAGAACGCAUGAAACGAA	1586
	GAUAAAGACACGAUUGAAA	1587
	GCUGAGAGGUCUAUUUAAA	1588
CLCA1	Sense	
	GAACAACAAUGGCUAUGAA	1589
	GUACAUACCUGGCUGGAUU	1590

GAACAGCUCACAAGUAUAU	1591
GGAAACGUGUGUCUAUAUU	1592

SLC6A1

Sense	
GGAGGUGGGAGGACAGUUA	1593
UCACAGCCCUGGUGGAUGA	1594
GAAGCUGGCUCCUAUGUUC	1595
GGUCAACACUACCAACAUG	1596

SLC6A2

Sense	
GAACACAAGGUCAACAUUG	1597
AGAAGGAGCUGGCCUAGUG	1598
CGGAAACUCUUCACAUUUG	1599
CAACAAAUUUGACAACAAC	1600

SLC21A2

Sense	
GUACAUCUCCAUCUUAUUU	1601
GGAAGUGGCUGAGUUAUUA	1602
GAAGGGAGGCUCAAUGUAA	1603
GAAGGAAGUGGCUGAGUUA	1604

SLC21A3

Sense	
GUAGAAACAGGAGCUAUUA	1605
CAAGAUUACUGUCAAACAA	1606
GCACAAGAGUAUUUGGUAA	1607
GCAAAUGUCCCUUCUGUAU	1608
GCAUGACUCCUAUAUAAUA	1609
AAACAGCAAUUUCCCUUAA	1610
GAAAAUGCCUCUUCAGGAA	1611

SLC28A1

Sense	
GUUCAUCGCUCUCCUCUUU	1612
GGAUCAAGCUGUUUCUGAA	1613
GGACUGCAGUUUGUACUUG	1614
GAGUGAAACUGACCUAUGG	1615

SLC29A1

Sense	
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GAAAGCCACUCUAUCAAAG	1617
GAAACCAGGUGCCUUCAGA	1618
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SLC26A1

Sense	
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GGGUUGACAUCUUAUUUGA	1621
GCACGAGGGUCUCUGUGUU	1622
GGCCAUCGCCUACUCAUUG	1623
CAACACCCAUGGCAAUUAA	1624
GAGGAAAGAUCUUGCUGAU	1625
GAGCAAGCGUCCUCCAAAU	1626
GCAACACCCAUGGCAAUUA	1627

SLC26A2

Sense	
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ACAAGAACCUUCAGACUAA	1629
GAAGGUAGAUAGAAGAAUG	1630

	GUAUUGAACUGUACUGUAA	1631

SLC4A4	Sense	
	GCAAUUCUCUUCAUUUAUC	1632
	GGAAAGAUGUCCACUGAAA	1633
	GGACAAAGCCUUCUUCAAU	1634
	GGAAUGGGAUCCAGCAAUU	1635

GLRA1	Sense	
	UGAAAGCCAUUGACAUUUG	1636
	CAGACACGCUGGAGUUUAA	1637
	CAAUAGCGCUUUCUGGUUU	1638
	GCAGGUAGCAGAUGGACUA	1639

KLK1	Sense	
	UCAGAGUGCUGUCUUAUGU	1640
	CAACUUGUUUGACGACGAA	1641
	UGACAGAGCCUGCUGAUAC	1642
	AGGCGGCUCUGUACCAUUU	1643

ADAM2	Sense	
	GAAACAUGCUGUGAUAUUG	1644
	GCAGAUGUUUCCUUAUAUA	1645
	CAACAGAGAUGCCAUGAUA	1646
	GAAAGGCGCUACAUUGAGA	1647

XPNPEP1	Sense	
	GACCUGAGCUUCCCAACAA	1648
	GCGACUGGCUCAACAAUUA	1649
	GAGAUUGCGUGGCUAUUUA	1650
	GACAGCAACUGGACACUUA	1651

GZMA	Sense	
	GGAAGAGACUCGUGCAAUG	1652
	GGAACCAUGUGCCAAGUUG	1653
	GAAGUAACUCCUCAUUCAA	1654
	GAACUCCUAUAGAUUUCUG	1655

CMKLR1	Sense	
	CAUAGAAGCUUUACCAAGA	1656
	GAAUGGAGGAUGAAGAUUA	1657
	GGUCAAUGCUCUAAGUGAA	1658
	GAGAGGACUUCUAUGAAUG	1659

CLN3	Sense	
	CAUCAUGCCUUCUGAAUAA	1660
	CAACAGCUCAUCACGAUUU	1661
	GCAACAACUUCUCUUAUGU	1662
	GGUCUUCGCUAGCAUCUCA	1663

CALCR	Sense	
	GGACCUAGCUGUUGUAAAG	1664
	GAAAGACCAUGCAUUUAAA	1665
	GCAGGAAGAUGUAUGCUUU	1666
	GAAUAAACCAGUAUCGUUA	1667

	Sense	
OXTR	GGACCCAGAUAUCCAAAUA	1668
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	GAAUAUAGAUUAGCGUUUG	1670
	GAUGAGGCAUGACUACUAA	1671

	Sense	
EDG4	GCGAGUCUGUCCACUAUAC	1672
	GAGAACGGCCACCCACUGA	1673
	GAACGGCCACCCACUGAUG	1674
	GGUCAAUGCUGCUGUGUAC	1675

	Sense	
EDG5	UCCAGGAACACUAUAAUUA	1676
	GUGACCAUCUUCUCCAUCA	1677
	CAUCCUCUGUUGCGCCAUU	1678
	CCAACAAGGUCCAGGAACA	1679

	Sense	
EDG7	ACACUGAUACUGUCGAUGA	1680
	AAUAGGAGCAACACUGAUA	1681
	CAGCAGGAGUUACCUUGUU	1682
	GGACACCCAUGAAGCUAAU	1683

	Sense	
PTCH	GCACAGAACUCCACUCAAA	1684
	GGACAGCAGUUCAUUGUUA	1685
	GAGAAGAGGCUAUGUUUAA	1686
	GGACAAACUUCGACCCUUU	1687

	Sense	
SMO	UCGCUACCCUGCUGUUAUU	1688
	GCUACAAGAACUACCGAUA	1689
	CAAGAAAGCUUCCUUCAAC	1690
	GAGAAGAAAUACAGUCAAU	1691

	Sense	
CASP3	CAAUAUAUCUGAAGAGCUA	1692
	GAACUGGACUGUGGCAUUG	1693
	GUGAGAAGAUGGUAUAUUU	1694
	GAGGGUACUUUAAGACAUA	1695

	Sense	
CASP6	CAUGAGGUGUCAACUGUUA	1696
	GAAGUGAAAUGCUUUAAUG	1697
	AAAUAUGGCUCCUCCUUAG	1698
	GCAAUCACAUUUAUGCAUA	1699
	CAACAUAACUGAGGUGGAU	1700
	CAUGGUACAUUCAAGAUUU	1701

	Sense	
CASP7	GAACUCUACUUCAGUCAAU	1704
	GGGCAAAUGCAUCAUAAUA	1703
	CAACAGAGGGAGUUUAAUA	1704
	GAACAAAGCCACUGACUGA	1705

CASP8

Sense	
GAAGUGAACUAUGAAGUAA	1706
CAACAAGGAUGACAAGAAA	1707
GGACAAAGUUUACCAAAUG	1708
GAGGGUCGAUCAUCUAUUA	1709
GAAUAUAGAGGGCUUAUGA	1710
CAACGACUAUGAAGAAUUC	1711
GAAGUGAGCAGAUCAGAAU	1712
GAGGAAAUCUCCAAAUGCA	1713

CASP9

Sense	
CCAGGCAGCUGAUCAUAGA	1714
UCUCAGGUGUUGCCAAAUA	1715
GAACAGCUGUAAUCUAUGA	1716
CCACUGGUCUGUAGGGAUU	1717

DVL1

Sense	
UCGUAAAGCUGUUGAUAUC	1718
GAGGAGAUCUUUGAUGACA	1719
GUAAAGCUGUUGAUAUCGA	1720
GAUCGUAAAGCUGUUGAUA	1721

DVL2

Sense	
AGACGAAGGUGAUUUACCA	1722
UGUGAGAGCUACCUAGUCA	1723
GAAGAAAUUUCAGAUGACA	1724
UAAUAGGCAUUUCCUCUUU	1725

PTEN

Sense	
GUGAAGAUCUUGACCAAUG	1726
GAUCAGCAUACACAAAUUA	1727
GAAUGAACCUUCUGCAACA	1728
GGCGCUAUGUGUAUUAUUA	1729

PDK1

Sense	
GUACAAAGCUGGUAUAUCC	1730
GAAAGACUCCCAGUGUAUA	1731
GGAAGUCCAUCUCAUCGAA	1732
CCAAAGACAUGACGACGUU	1733

PDK2

Sense	
GUAAAGAGGAGACUGAAUG	1734
GGUCUGUGAUGGUCCCUAA	1735
CAAAGAUGCCUACGACAUG	1736
GGGCGAUGCCUGAGGGUUA	1737

PPP2CA

Sense	
UCACACAAGUUUAUGGUUU	1738
CAACAGCCGUGACCACUUU	1739
UAACCAAGCUGCAAUCAUG	1740
GAACUUGACGAUACUCUAA	1741

CTNNA1

Sense	
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AAGCAGAUGUGCAUGAUUA	1743
UCUAAUAACUGCAGUGUUU	1744

	GUAAAGGGCCCUCUAAUAA	1745

CTNNA2	Sense	
	GAAAGAAUAUGCCCAAGUU	1746
	GAAGAAGAAUGCCACAAUG	1747
	GCAGGAAGAUUAUGAUGUG	1748
	AAAGAAAGCCCAUGUACUA	1749

HSPCA	Sense	
	GGGAAAGAGCUGCAUAUUA	1750
	GCUUAGAACUCUUUACUGA	1751
	UAUAAGAGCUUGACCAAUG	1752
	GCAGAUAUCUCUAUGAUUG	1753

DCTN2	Sense	
	CAACUCAUGUCCAAUACUG	1754
	GGAAUGAGCCAGAUGUUUA	1755
	GGAGACAGCUGUACGUUGU	1756
	UCCAAGAGCUGACAACUGA	1757

CD2	Sense	
	GUAAGGAGAAGCAAUAUAA	1758
	AAGAUGAGCUUUCCAUGUA	1759
	GGACAUCUAUCUCAUCAUU	1760
	GACAAGAGCCCACAGAGUA	1761

BAD	Sense	
	GUACUUCCCUCAGGCCUAU	1762
	GCUGUGCCUUGACUACGUA	1763
	GUACUUCCCUCAGGCCUAU	1764
	GGUCAGGUGCCUCGAGAUC	1765

SMAC	Sense	
	CAGCGUAACUUCAUUCUUC	1766
	UAACUUCAUUCUUCAGGUA	1767
	CAGCUGCUCUUACCCAUUU	1768
	GAUUGAAGCUAUUACUGAA	1769
	UAGAAGAGCUCCGUCAGAA	1770
	CCACAUAUGCGUUGAUUGA	1771
	GCGCAGGGCUCUCUACCUA	1772

MAP3K5	Sense	
	GAACAGCCUUCAAAUCAAA	1773
	GAUGUUCUCUACUAUGUUA	1774
	GCAAAUACUGGAAGGAUUA	1775
	CAGGAAAGCUCGUAAUUUA	1776

PVR	Sense	
	CCACACGGCUGACCUCAUA	1777
	CAGCAGAAUUCCUCUUAUA	1778
	GCAGAAUUCCUCUUAUAAA	1779
	GAUCGGGAUUUAUUUCUAU	1780

ERBB2	Sense	
	UGUGGGAGCUGAUGACUUU	1781
	UCACAGAGAUCUUGAAAGG	1782

UGGAAGAGAUCACAGGUUA	1783
GCUCAUCGCUCACAACCAA	1784

SOS1

Sense	
GAGCACCACUUCUAUGAUU	1785
CAAAGAAGCUGUUCAAUAU	1786
UGAAAGCCCUCCCUUAUUA	1787
GAAAUAGCAUGGAGAAGGA	1788

BRCA1

Sense	
CCAUACAGCUUCAUAAAUA	1789
GAAGAGAACUUAUCUAGUG	1790
GAAGUGGGCUCCAGUAUUA	1791
GCAAGAUGCUGAUUCAUUA	1792
GAAGUGGGCUCCAGUAUUA	1793
GAACGGACACUGAAAUAUU	1794
GCAGAUAGUUCUACCAGUA	1795

CDKN1A

Sense	
GAACAAGGAGUCAGACAUU	1796
AAACUAGGCGGUUGAAUGA	1797
GAUGGAACUUCGACUUUGU	1798
GUAAACAGAUGGCACUUUG	1799

CDKN1B

Sense	
GGAAUGGACAUCCUGUAUA	1800
GGAGAAAGAUGUCAAACGU	1801
GAAUGGACAUCCUGUAUAA	1802
GUAAACAGCUCGAAUUAAG	1803

SLC2A4

Sense	
CAGAUAGGCUCCGAAGAUG	1804
AGACUCAGCUCCAGAAUAC	1805
GAUCGGUUCUUUCAUCUUC	1806
CAGGAUCGGUUCUUUCAUC	1807

NOS2A

Sense	
CCAGAUAAGUGACAUAAGU	1808
UAAGUGACCUGCUUUGUAA	1809
GAAGAGAGAUUCCAUUGAA	1810
UGAAAGAGCUCAACAACAA	1811

FRAP1

Sense	
GAGCAUGCCGUCAAUAAUA	1812
CAAGAGAACUCAUCAUAAG	1813
CCAAAGUGCUGCAGUACUA	1814
UAAGAAAGCUAUCCAGAUU	1815

FKBP1A

Sense	
GAAACAAGCCCUUUAAGUU	1816
GAAUUACUCUCCAAGUUGA	1817
CAGCACAAGUGGUAGGUUA	1818
GUUGAGGACUGAAUUACUC	1819
GAUGGCAGCUGUUUAAAUG	1820
GAGUAUCCUUUCAGUGUUA	1821

TNFRSF 1A

Sense	
CAAAGGAACCUACUUGUAC	1822
GGAACCUACUUGUACAAUG	1823
GAACCUACUUGUACAAUGA	1824
GAGUGUGUCUCCUGUAGUA	1825

IL1R1

Sense	
GGACAAGAAUCAAUGGAUA	1826
GAACAAGCCUCCAGGAUUC	1827
GGACUUGUGUGCCCUUAUA	1828
GAACACAAAGGCACUAUAA	1829

IRAK1

Sense	
CGAAGAAAGUGAUGAAUUU	1830
GCUCUUUGCCCAUCUCUUU	1831
UGAAAGACCUGGUGGAAGA	1832
GCAAUUCAGUUUCUACAUC	1833

TRAF2

Sense	
GAAGACAGAGUUAUUAAAC	1834
UCACGAAGACAGAGUUAUU	1835
AGACAGAGUUAUUAAACCA	1836
CACGAAGACAGAGUUAUUA	1837
GCUGAAGCCUGUCUGAUGU	1838

TRAF6

Sense	
CAAAUGAUCUGAGGCAGUU	1839
GUUCAUAGUUUGAGCGUUA	1840
GGAGAAACCUGUUGUGAUU	1841
GGACAAAGUUGCUGAAAUC	1842
CAAAUGAUCUGAGGCAGUU	1843
GGAGAAACCUGUUGUGAUU	1844
GGACAAAGUUGCUGAAAUC	1845
GUUCAUAGUUUGAGCGUUA	1846

TRADD

Sense	
UGAAGCACCUUGAUCUUUG	1847
GGGCAGCGCAUACCUGUUU	1848
GAGGAGCGCUGUUUGAGUU	1849
GGACGAGGAGCGCUGUUUG	1850
GAGGAGCGCUGUUUGAGUU	1851
GGAUGUCUCUCUCCUCUUU	1852
GCUCACUCCUUUCUACUAA	1853
UGAAGCACCUUGAUCUUUG	1854

FADD

Sense	
GCACAGAUAUUUCCAUUUC	1855
GCAGUCCUCUUAUUCCUAA	1856
GAACUCAAGCUGCGUUUAU	1857
GGACGAAUUGAGAUAAUAU	1858

IKBKE

Sense	
UAAGAACACUGCUCAUGAA	1859
GAGGCAUCCUGAAGCAUUA	1860
GAAGGCGGCUGCAGAACUG	1861

	GGAACAAGGAGAUCAUGUA	1862

IKBKG	Sense	
	CUAUCGAGGUCGUUAAAUU	1863
	GAAUGCAGCUGGAAGAUCU	1864
	GCGGCGAGCUGGACUGUUU	1865
	CCAGACCGAUGUGUAUUUA	1866

TNFRSF 5	Sense	
	GGUCUCACCUCGCUAUGGU	1867
	GAAAGCGAAUUCCUAGACA	1868
	GCACAAACAAGACUGAUGU	1869
	GAAGGGCACCUCAGAAACA	1870
	UCUCCCAACUUGUAUUAAA	1871

RELA	Sense	
	UCAAGUGUCUUCCAUCAUG	1872
	UCAAGUGCCUUAAUAGUAG	1873
	GGAGUACCCUGAGGCUAUA	1874
	GAUGAGAUCUUCCUACUGU	1875

ARHA	Sense	
	GAGCUGGGCUAAGUAAAUA	1876
	GACCAAAGAUGGAGUGAGA	1877
	GGAAGAAACUGGUGAUUGU	1878
	GGCUGUAACUACUUUAUAA	1879

CDC42	Sense	
	GGACAUUUGUUUGCCAUUU	1880
	GGAGAACCAUAUACUCUUG	1881
	GAACCAAUGCUUUCUCAUG	1882
	GAAGACCUGUUAUGUAGAG	1883
	GAUCAAGAAUUGCAAUAUC	1884
	GAAAAGGGUGACCUAGUA	1885
	UGACAAACCUUAUGGAAAA	1886

ROCK1	Sense	
	GGAAUGAGCUUCAGAUGCA	1887
	GGACACAGCUGUAAGAUUG	1888
	GACAAGAGAUUACAGAUAA	1889
	GAAGAAACAUUCCCUAUUC	1890

PAK1	Sense	
	GAGGGUGGUUUAUGAUUAA	1891
	CAACAAAGAACAAUCACUA	1892
	GAAGAAAUAUACACGGUUU	1893
	UACAUGAGCUUUACAGAUA	1894

PAK2	Sense	
	GGUAGGAGAUGAAUUGUUU	1895
	AGAAGGAACUGAUCAUUAA	1896
	CUACAGACCUCCAAUAUCA	1897
	GAAACUGGCCAAACCGUUA	1898

PAK3	Sense	

GAUUAUCGCUGCAAAGGAA	1899
GAGAGUGCCUGCAAGCUUU	1900
GACAAGAGGUGGCCAUAAA	1901
UUAAAUCGCUGUCUUGAGA	1902

PAK4

Sense	
ACUAAGAGGUGAACAUGUA	1903
GAUCAUGAAUGUCCGAAGA	1904
GAUGAGACCCUACUACUGA	1905
CAGCAAAGGUGCCAAAGAU	1906

PAK6

Sense	
UAAAGGCAGUUGUCCACUA	1907
GAAGGGACCUGCUUUCUUG	1908
GCAAAGACGUCCCUAAGAG	1909
CCAAUGGGCUGGCUGCAAA	1910

PAK7

Sense	
GAGCACGGCUUUAAUAAGU	1911
CAAACUCCGUUAUGAUAUA	1912
GGAUAAAGUUGUCUGAUUU	1913
GGAAAUGCCUCCAUAAAUA	1914

HDAC1

Sense	
GGACAUCGCUGUGAAUUGG	1915
AGAAAGAAGUCACCGAAGA	1916
GGACAAGGCCACCCAAUGA	1917
CCACAGCGAUGACUACAUU	1918

HDAC2

Sense	
GCUGUUAAAUUAUGGCUUA	1919
GCAAAGAAAGCUAGAAUUG	1920
CAUCAGAGAGUCUUAUAUA	1921
CCAAUGAGUUGCCAUAUAA	1922

CREBBP

Sense	
GGCCAUAGCUUAAUUAAUC	1923
GCACAGCCGUUUACCAUGA	1924
GGACAGCCCUUUAGUCAAG	1925
GAACUGAUUCCUGAAAUAA	1926

BTRC

Sense	
CACAUAAACUCGUAUCUUA	1927
GAGAAGGCACUCAAGUUUA	1928
AGACAUAGUUUACAGAGAA	1929
GCAGAGAGAUUUCAUAACU	1930

RIPK2

Sense	
GAACAUACCUGUAAAUCAU	1931
GGACAUCGACCUGUUAUUA	1932
UAAAUGAACUCCUACAUAG	1933
GGAAUUAUCUCUGAACAUA	1934

VAV1

Sense	
GCAGAAAUACAUCUACUAA	1935
GCUAUGAGCUGUUCUUCAA	1936

CGACAAAGCUCUACUCAUC	1937
GCUCAACCCUGGAGACAUU	1938

VAV2

Sense	
GGACAAGACUCGCAGAUUU	1939
GCUGAGCGCUUUGCAAUAA	1940
CAAGAAGUCUCACGGGAAA	1941
UCACAGAGGCCAAGAAAUU	1942

GRB2

Sense	
UGGAAGCCAUCGCCAAAUA	1942
CAUCAGUGCAUGACGUUUA	1943
UGAAUGAGCUGGUGGAUUA	1944
UGCCAAAACUUACCUAUAA	1945

PLCG1

Sense	
GAGCUGCACUCCAAUGAGA	1946
GAAACCAAGCCAUUAAUGA	1947
CCAAGGAGCUACUGACAUU	1948
AGAGAAACAUGGCCCAAUA	1949

ITGB1

Sense	
CCACAGACAUUUACAUUAA	1950
GAAGGGAGUUUGCUAAAUU	1951
GAACAGAUCUGAUGAAUGA	1952
CAAGAGAGCUGAAGACUAU	1953

ITGA4

Sense	
GCAUAUAUAUUCAGCAUUG	1954
CAACUUGACUGCAGUAUUG	1955
GAACUUAACUUUCCAUGUU	1956
GACAAGACCUGUAGUAAUU	1957

STAT1

Sense	
AGAAAGAGCUUGACAGUAA	1958
GGAAGUAGUUCACAAAAUA	1959
UGAAGUAUCUGUAUCCAAA	1960
GAGCUUCACUCCCUUAGUU	1961

KRAS2

Sense	
UAAGGACUCUGAAGAUGUA	1962
GACAAAGUGUGUAAUUAUG	1963
GCUCAGGACUUAGCAAGAA	1964
GAAACUGAAUACCUAAGAU	1965
GAAACUGAAUACCUAAGAU	1966
UAAGGACUCUGAAGAUGUA	1967
GACAAAGUGUGUAAUUAUG	1968
GCUCAGGACUUAGCAAGAA	1969

HRAS

Sense	
CCAUCCAGCUGAUCCAGAA	1970
GAACCCUCCUGAUGAGAGU	1971
GAGGACAUCCACCAGUACA	1972

BRAF

Sense	
GAUUAGAGACCAAGGAUUU	

CCACUGAUGUGUGUUAAUU	1973
CAAUAGAACCUGUCAAUAU	1974
GAAGACAGGAAUCGAAUGA	1975

ELK1

Sense	
GAUGUGAGUAGAAGAGUUA	1975
GGAAGAAUUUGUACCAUUU	1976
GAACGACCUUUCUUUCUUU	1977
GGAGUCAUCUCUUCCUAUA	1978

RALGDS

Sense	
GGAGAAGCCUCACCUCUUG	1979
GCAGAAAGGACUCAAGAUU	1980
GAGAACAACUACUCAUUGA	1981
GAACUUCUCGUCACUGUAU	1982

PRKCA

Sense	
GGAUUGUUCUUUCUUCAUA	1983
GAAGGGUUCUCGUAUGUCA	1984
GAAGAAGGAUGUGGUGAUU	1985
GGACUGGGAUCGAACAACA	1986

MAP2K4

Sense	
GGACAGAAGUGGAAAUAUU	1987
UCAAAGAGGUGAACAUUAA	1988
GACCAAAUCUCAGUUGUUU	1989
GGAGAAUGGUGCUGUUUAA	1990

MAP2K7

Sense	
GAAGAGACCAAAGUAUAAU	1991
GAAGACCGGCCACGUCAUU	1992
GGAAGAGACCAAAGUAUAA	1993
GCAUUGAGAUUGACCAGAA	1994
UGAGAGAACGAGAAAGUUG	1995
GUGAAACCCUGUCUGCAUU	1996
GGAUCUCUCUCAACAACUA	1997
ACAACUAGGUGAACACAUA	1998

MAPK8

Sense	
UCACAGUCCUGAAACGAUA	1999
GAUUGGAGAUUCUACAUUC	2000
GCUCAUGGAUGCAAAUCUU	2001
GAAGCUAAGCCGACCAUUU	2002

MAPK9

Sense	
AAAGAGAGCUUAUCGUGAA	2003
GAUGAUAGGUUAGAAAUAG	2004
ACAAAGAAGUCAUGGAUUG	2005
GGAGCUGGAUCAUGAAAGA	2006

AIF1

Sense	
GAAAAGGGAUGAUGGGAUU	2007
CCUAGACGAUCCCAAAUAU	2008
GAGCCAAACCAGGGAUUUA	2009
UGAAACGAAUGCUGGAGAA	2010
UCACUCACCCAGAGAAAUA	2011

CCAAGAAAGCUAUCUCUGA	2012
AGACUCACCUAGAGCUAAA	2013

BBC3

Sense	
CCUGGAGGGUCCUGUACAA	2014
GAGCAAAUGAGCCAAACGU	2015
GGAGGGUCCUGUACAAUCU	2016
GACUUUCUCUGCACCAUGU	2017

BCL2L1

Sense	
CCAGGGAGCUUGAAAGUUU	2018
AAAGUGCAGUUCAGUAAUA	2019
GAGAAUCACUAACCAGAGA	2020
GAGCCCAUCCCUAUUAUAA	2021

BCL2L11

Sense	
GAGACGAGUUUAACGCUUA	2022
AAAGCAACCUUCUGAUGUA	2023
CCGAGAAGGUAGACAAUUG	2024
GCAAAGCAACCUUCUGAUG	2025
AGACAGAGCCACAAGGUAA	2026
GCAAGGAGGUUAGAGAAAU	2027
CAAGGAGGUUAGAGAAAUA	2028
UCUUACGACUGUUACGUUA	2029

BID

Sense	
GAAGACAUCAUCCGGAAUA	2030
CAACAGCGUUCCUAGAGAA	2031
GAAAUGGGAUGGACUGAAC	2032
ACGAUGAGCUGCAGACUGA	2033

BIRC2

Sense	
GAAAGAAGCCUGCAUAUAA	2034
GAAAUUGACUCUACAUUGU	2035
ACAAAUAGCACUUAGGUUA	2036
GAAUACACCUGUGGUUAAA	2037

BIRC3

Sense	
GGAGAUGCCUGCCAUUAAA	2038
UCAAUGAUCUUGUGUUAGA	2039
GAAAGAACAUGUAAAGUGU	2040
GAAGAAAGAACAUGUAAAG	2041

BIRC4

Sense	
GUAGAUAGAUGGCAAUAUG	2042
GAGGAGGGCUAACUGAUUG	2043
GAGGAACCCUGCCAUGUAU	2044
GCACGGAUCUUUACUUUUG	2045

BIRC5

Sense	
GGCGUAAGAUGAUGGAUUU	2046
GCAAAGGAAACCAACAAUA	2047
GCACAAAGCCAUUCUAAGU	2048
CAAAGGAAACCAACAAUAA	2049

BRCA1

Sense	

CCAUACAGCUUCAUAAAUA	2050
GAAGAGAACUUAUCUAGUG	2051
GAAGUGGGCUCCAGUAUUA	2052
GCAAGAUGCUGAUUCAUUA	2053
CCAUACAGCUUCAUAAAUA	2054

CARD4

Sense	
GAAAGUUAAUGUCAAGGAA	2055
GAGCAACACUGGCAUAACA	2056
UAACAGAGAUUUGCCUAAA	2057
GCGAAGAGCUGACCAAAUA	2058

CASP10

Sense	
CAAAGGGUUUCUCUGUUUA	2059
GAAAUGACCUCCCUAAGUU	2060
GAAGGCAGCUGGUAUAUUC	2061
GACAUGAUCUUCCUUCUGA	2062
GCACUCUUCUGUUCCCUUA	2063

CASP2

Sense	
GUAUUAAACUCUCCUUUGA	2064
GCAAGGAGAUGUCUGAAUA	2065
CAACUUCCCUGAUCUUUAA	2066
GCUCAAAGAUGUAAUGUAG	2067

CDKN1A

Sense	
GAACAAGGAGUCAGACAUU	2068
AAACUAGGCGGUUGAAUGA	2069
GAUGGAACUUCGACUUUGU	2070
GUAAACAGAUGGCACUUUG	2071

CFLAR

Sense	
GAUGUGUCCUCAUUAAUUU	2072
GAAGAGAGAUACAAGAUGA	2073
GAGCAUACCUGAAGAGAGA	2074
GCUAUGAAGUCCAGAAAUU	2075

CLK2

Sense	
GUGAAUAUGUGAAAUAGUG	2076
AAAGCAUGCUAGAGUAUGA	2077
UUAAGAAUGUGGAGAAGUA	2078
GAUAACAAGCUGACACAUA	2079

CLSPN

Sense	
GGACGUAAUUGAUGAAGUA	2080
GCAGAUGGGUUCUUAAAUG	2081
CAAAUGAGGUUGAGGAAAU	2082
GGAAAUACCUGGAGGAUGA	2083

CSNK2A 1

Sense	
GAUCCACGUUUCAAUGAUA	2084
GCAUUUAGGUGGAGACUUC	2085
GAUGUACGAUUAUAGUUUG	2086
UGAAUUAGAUCCACGUUUC	2087

CTNNB1

Sense	
GCACAAGAAUGGAUCACAA	2088
GCUGAAACAUGCAGUUGUA	2089
GUACGUACCAUGCAGAAUA	2090
GAACUUGCAUUGUGAUUGG	2091

CXCR4

Sense	
GAAGCAUGACGGACAAGUA	2092
GAACAUUCCAGAGCGUGUA	2093
GUUCUUAGUUGCUGUAUGU	2094
CAUCAUGGUUGGCCUUAUC	2095

CXCR6

Sense	
GGAACAAACUGGCAAAGCA	2096
GAUCAGAGCAGCAGUGAAA	2097
GGGCAAAACUGAAUUAUAA	2098
GAUCUCAGGUUCUCCUUGA	2099

DAXX

Sense	
CUACAGAUCUCCAAUGAAA	2100
GCUACAAGCUGGAGAAUGA	2101
GGAAACAGCUAUGUGGAAA	2102
GGAGUUGGAUCUCUCAGAA	2103

GAS41

Sense	
GUAGUAAGCUAAACUGAAA	2104
GACAAUAUGUUCAAGAGAA	2105
GACAACAUCUCGUCAGCUA	2106
UAUAUGAUGUGUCCAGUAA	2107

GTSE1

Sense	
CAAAGAAGCUCACUUACUG	2108
GAACAGCCCUAAAGUGGUU	2109
GAACAUGGAUGACCCUAAG	2110
GGGCAAAGCUAAAUCAAGU	2111

HDAC3

Sense	
GGAAAGCGAUGUGGAGAUU	2112
CCAAGACCGUGGCCUAUUU	2113
AAAGCGAUGUGGAGAUUUA	2114
GUGAGGAGCUUCCCUAUAG	2115

HDAC5

Sense	
GAAUUCCUCUUGUCGAAGU	2116
GUUAUUAGCACCUUUAAGA	2117
GGAGGGAGGCCAUGACUUG	2118
CAGGAGAGCUCAAGAAUGG	2119
GGAUAUGGAUUUCAGUUAA	2120
GGAAGUCGGUGCCUUGGUU	2121
GGAAGGAGAGGACUGGUUU	2122

HEC

Sense	
GCAGAUACUUGCACGGUUU	2123
GAGUAGAACUAGAAUGUGA	2124
GCGAAUAAAUCAUGAAAGA	2125
GAAGAUGGAAUUAUGCAUA	2126

HIST1H2AA	Sense	
	GGCAAUGCGUCUCGCGAUA	2127
	GAUCCGCAAUGAUGAGGAA	2128
	GCAAUGCGUCUCGCGAUAA	2129
	GAGGAACUCAAUAAGCUUU	2130

LMNB1	Sense	
	AAUAGAAGCUGUGCAAUUA	2131
	CAACUGACCUCAUCUGGAA	2132
	GAAGGAAUCUGAUCUUAAU	2133
	GGGAAGGGUUUCUCUAUUA	2134

LMNB2	Sense	
	GGAGGUUCAUUGAGAAUUG	2134
	GGCAAUAGCUCACCGUUUA	2135
	CAAAUACGCUUAGCUGUGU	2136
	GGAGAUCGCCUACAAGUUC	2137

MYB	Sense	
	GCAGAAACACUCCAAUUUA	2138
	GUAAAUACGUGAAUGCAUU	2139
	GCACUGAACUUUUGAGAUA	2140
	GAAGAACAGUCAUUUGAUG	2141

MYT1	Sense	
	GAGGUGAGCUGUUAAAUCA	2142
	GCAGGGUGAUUUCCUAAUA	2143
	GGGAGAAGAUAUUUAAUUG	2144
	CAACUUCUCUCCUGAACUU	2145

NFKBIB	Sense	
	GGACACGGCACUGCACUUG	2146
	GCACUUGGCUGUGAUUCAU	2148
	GAGACGAGGGCGAUGAAUA	2149
	CAUGAACCCUUCCUGGAUU	2150

NFKBIA	Sense	
	GAACAUGGACUUGUAUAUU	2151
	GAUGUGGGGUGAAAAGUUA	2152
	GGACGAGAAAGAUCAUUGA	2153
	AGGACGAGCUGCCCUAUGA	2154

NFKBIE	Sense	
	GAAGGGAAGUUUCAGUAAC	2155
	GGAAGGGAAGUUUCAGUAA	2156
	GGAAACUGCUGCUGUGUAC	2157
	GAACCAACCACUCAUGGAA	2158

NUMA1	Sense	
	GGGAACAGUUUGAAUAUAA	2159
	GCAGUAGCCUGAAGCAGAA	2160
	CGAGAAGGAUGCACAGAUA	2161
	GCAAGAGGCUGAGAGGAAA	2162

NUP153	Sense	

	GAAGACAAAUGAAAGCUAA	2163
	GAUAAAGACUGCUGUUAGA	2164
	GAGGAGAGCUCUAAUAUUA	2165
	GAGGAAGCCUGAUUAAAGA	2166

OPA1	Sense	
	GAAAGAGCAUGAUGACAUA	2167
	GAGGAGAGCUCUAUUAUGU	2168
	GAAACUGAAUGGAAGAAUA	2169
	AAAGAAGGCUGUACCGUUA	2170

PARVA	Sense	
	CUACAUGUCUUUGCUCUUA	2171
	GCUAAGUCCUGUAAGAAUA	2172
	CAAAGGCAAUGUACUGUUU	2173
	GAACAAUGGUGGAUCCAAA	2174

PIK3CG	Sense	
	AAGUUCAGCUUCUCUAUUA	2175
	GAAGAAAUCUCUGAUGGAU	2176
	GAACACCUUUACUCUAUAA	2177
	GCAUGGAGCUGGAGAACUA	2178

PRKDC	Sense	
	GAUGAAAGCUCUAAAGAUG	2179
	GAAAGGAGGUUCUAAACUA	2180
	GGAAGAAGCUCAUUUGAUU	2181
	GCAAAGAGGUGGCAGUUAA	2182

RASA1	Sense	
	GGAAGAAGAUCCACAUGAA	2183
	GAACAUACUUUCAGAGCUU	2184
	GAACAAUCUUUGCUGUAUA	2185
	UAACAGAACUGCUUCAACA	2186

SLC9A1	Sense	
	GAAGAGAUCCACACACAGU	2187
	UCAAUGAGCUGCUGCACAU	2188
	GAAGAUAGGUUUCCAUGUG	2189
	GAAUUACCCUUCCUCAUCU	2190

TEGT	Sense	
	CUACAGAGCUUCAGUGUGA	2191
	GAACAUAUUUGAUCGAAAG	2192
	GAGCAAACCUAGAUAAGGA	2193
	GCAUUGAUCUCUUCUUAGA	2194

TERT	Sense	
	GGAAGACAGUGGUGAACUU	2195
	GCAAAGCAUUGGAAUCAGA	2196
	GAGCUGACGUGGAAGAUGA	2197
	GAACGGGCCUGGAACCAUA	2198

TNFRSF 6	Sense	
	GAUACUAACUGCUCUCAGA	2199

GAAAGAAUGGUGUCAAUGA	2200
UCAAUAAUGUCCCAUGUAA	2201
UCAUGAAUCUCCAACCUUA	2202
GAUGUUGACUUGAGUAAAU	2203

TOP1

Sense	
GAAAGGAAAUGACUAAUGA	2204
GAAGAAGGCUGUUCAGAGA	2205
GGAAGUAGCUACGUUCUUU	2206
GGACAUAAGUGGAAAGAAG	2207

TOP2A

Sense	
GAAAGAGUCCAUCAGAUUU	2208
CAAACUACAUUGGCAUUUA	2209
AAACAGACAUGGAUGGAUA	2210
CGAAAGGAAUGGUUAACUA	2211

TOP3A

Sense	
CCAGAAAUCUUCCACAGAA	2212
GAAACUAUCUGGAUGUGUA	2213
CCACAAAGAUGGUAUCGUA	2214
GGAAAUGGCUGUGGUAACA	2215

TOP3B

Sense	
GAGACAAGAUGAAGACUGU	2216
GCACAUGGGCUGCGUCUUU	2217
CCAGUGCGCUUCAAGAUGA	2218
GAACAUCUGCUUUGAGGUU	2219

WEE1

Sense	
GGUAUUGCCUUGUGAAUUU	2220
GCAGAACAAUUACGAAUAG	2221
GUACAUAGCUGUUUGAAAU	2222
GCUGUAAACUUGUAGCAUU	2223

In addition, to identifying functional siRNA against gene families or pathways, it is possible to design duplexes against genes known to be involved in specific diseases. For example when dealing with human disorders associated with allergies, it will be beneficial to develop siRNA against a number of genes including but not limited to:

the interleukin 4 receptor gene

(SEQ. ID NO. 2224: UAGAGGUGCUCAUUCAUUU,

SEQ. ID NO. 2225: GGUAUAAGCCUUUCCAAGA,

SEQ. ID NO. 2225: ACACACAGCUGGAAGAAAU,

SEQ. ID NO. 2226: UAACAGAGCUUCCUUAGGU),

the Beta-arrestin-2

(SEQ. ID NO. 2227: GGAUGAAGGAUGACGACUA,
SEQ. ID NO. 2228: ACACCAACCUCAUUGAAUU,
SEQ. ID NO. 2229: CGAACAAGAUGACCAGGUA,
SEQ. ID NO. 2230: GAUGAAGGAUGACGACUAU,),

the interferon-gamma receptor 1 gene
(SEQ. ID NO. 2231: CAGCAUGGCUCUCCUCUUU,
SEQ. ID NO. 2232: GUAAAGAACUAUGGUGUUA,
SEQ. ID NO. 2233: GAAACUACCUGUUACAUUA,
SEQ. ID NO. 2234: GAAGUGAGAUCCAGUAUAA),

the matrix metalloproteinase MMP-9
(SEQ. ID NO. 2235: GGAACCAGCUGUAUUUGUU,
SEQ. ID NO. 2236: GUUGGAGUGUUUCUAAUAA,
SEQ. ID NO. 2237: GCGCUGGGCUUAGAUCAUU,
SEQ. ID NO. 2238: GGAGCCAGUUUGCCGGAUA),

the Slc11a1 (Nramp1) gene
(SEQ. ID NO. 2239: CCAAUGGCCUGCUGAACAA,
SEQ. ID NO. 2240: GGGCCUGGCUUCCUCAUGA,
SEQ. ID NO. 2241: GGGCAGAGCUCCACCAUGA,
SEQ. ID NO. 2242: GCACGGCCAUUGCAUUCAA),

SPINK5
(SEQ. ID NO. 2243: CCAACUGCCUGUUCAAUAA,
SEQ. ID NO. 2244: GGAUACAUGUGAUGAGUUU,
SEQ. ID NO. 2245: GGACGAAUGUGCUGAGUAU,
SEQ. ID NO. 2246: GAGCUUGUCUUAUUUGCUA,),

the CYP1A2 gene
(SEQ. ID NO. 2247: GAAAUGCUGUGUCUUCGUA,
SEQ. ID NO. 2248: GGACAGCACUUCCCUGAGA,
SEQ. ID NO. 2249: GAAGACACCACCAUUCUGA,
SEQ. ID NO. 2250: GGCCAGAGCUUGACCUUCA),

thymosin-beta4Y
(SEQ. ID NO. 2251: GGACAGGCCUGCGUUGUUU,
SEQ. ID NO. 2252: GGAAAGAGGAAGCUCAUGA,
SEQ. ID NO. 2253: GCAAACACGUUGGAUGAGU,
SEQ. ID NO. 2254: GGACUAUGCUGCCCUUUUG,

activin A receptor IB
(SEQ. ID NO. 2255: ACAAGACGCUCCAGGAUCU,
SEQ. ID NO. 2254: GCAACAGGAUCGACUUGAG,
SEQ. ID NO. 2255: GAAGCUGCGUCCCAACAUC,
SEQ. ID NO. 2256: GCAUAGGCCUGUAAUCGUA,
SEQ. ID NO. 2257: UCAGAGAGUUCGAGACAAA,
SEQ. ID NO. 2258: UGCGAAAGGUUGUAUGUGA,
SEQ. ID NO. 2259: GCAACAGGAUCGACUUGAG,
SEQ. ID NO. 2260: GAAUAGCGUUGUGUGUUAU,
SEQ. ID NO. 2261: UGAAUAGCGUUGUGUGUUA,
SEQ. ID NO. 2262: GGGAUCAGUUUGUUGAAUA,
SEQ. ID NO. 2263: GAGCCUGAAUCAUCGUUUA,),

ADAM33
(SEQ. ID NO. 2264: GGAAGUACCUGGAACUGUA,
SEQ. ID NO. 2265: GGACAGAGGGAACCAUUUA,
SEQ. ID NO. 2266: GGUGAGAGGUAGCUCCUAA,
SEQ. ID NO. 2267: AAAGACAGGUGGCCACUGA),

the TAP1 gene
(SEQ. ID NO. 2268: GAAAGAUGAUCAGCUAUUU,
SEQ. ID NO. 2269: CAACAGAACCAGACAGGUA,
SEQ. ID NO. 2270: UGAGAAAUGUUCAGAAUGU,
SEQ. ID NO. 2271: UACCUUCACUCGAAACUUA,

COX-2
(SEQ. ID NO. 2272: GAACGAAAGUAAAGAUGUU,

SEQ. ID NO. 2273: GGACUUAUGGGUAAUGUUA,
SEQ. ID NO. 2274: UGAAAGGACUUAUGGGUAA,
SEQ. ID NO. 2275: GAUCAGAGUUCACUUUCUU),

ADPRT
(SEQ. ID NO. 2276: GGAAAGAUGUUAAGCAUUU,
SEQ. ID NO. 2277: CAUGGGAGCUCUUGAAAUA,
SEQ. ID NO. 2278: GAACAAGGAUGAAGUGAAG,
SEQ. ID NO. 2279: UGAAGAAGCUCACAGUAAA,),

HDC
(SEQ. ID NO. 2280: CAGCAGACCUUCAGUGUGA,
SEQ. ID NO. 2281: GGAGAGAGAUGGUGGAUUA,
SEQ. ID NO. 2282: GUACAGAGCUGGAGAUGAA,
SEQ. ID NO. 2283: GAACGUCCCUUCAGUCUGU),

HnmT
(SEQ. ID NO. 2284: CAAAUUCUCUCCAAAGUUC,
SEQ. ID NO. 2285: GGAUAUAUCUGACUGCUUU,
SEQ. ID NO. 2286: GAGCAGAGCUUGGGAAAGA,
SEQ. ID NO. 2287: GAUAUGAGAUGUAGCAAAU),

GATA-3
(SEQ. ID NO. 2288: GAACUGCUUUCUUUCGUUU,
SEQ. ID NO. 2289: GCAGUAUCAUGAAGCCUAA,
SEQ. ID NO. 2290: GAAACUAGGUCUGAUAUUC,
SEQ. ID NO. 2291: GUACAGCUCCGGACUCUUC),

Gab2
(SEQ. ID NO. 2292: GCACAACCAUUCUGAAGUU,
SEQ. ID NO. 2293: GGACUUAGAUGCCCAGAUG,
SEQ. ID NO. 2294: GAAGGUGGAUUCUAGGAAA,
SEQ. ID NO. 2295: GGACUAGCCCUGCUGUUUA), and

STAT6
(SEQ. ID NO. 2296: GAUAGAAACUCCUGCUAAU,
SEQ. ID NO. 2297: GGACAUUUAUUCCCAGCUA,
SEQ. ID NO. 2298: GGACAGAGCUACAGACCUA,
SEQ. ID NO. 2299: GGAUGGCUCUCCACAGAUA).

In addition, rationally designed siRNA or siRNA pools can be directed against genes involved in anemia, hemophila or hypercholesterolemia. Such genes would include, but are not be limited to:

APOA5
(SEQ. ID NO. 2300: GAAAGACAGCCUUGAGCAA,
SEQ. ID NO. 2301: GGACAGGGAGGCCACCAAA,
SEQ. ID NO. 2302: GGACGAGGCUUGGGCUUUG,
SEQ. ID NO. 2303: AGCAAGACCUCAACAAUAU),

HMG-CoA reductase
(SEQ. ID NO. 2304: GAAUGAAGCUUUGCCCUUU,
SEQ. ID NO. 2305: GAACACAGUUUAGUGCUUU,
SEQ. ID NO. 2306: UAUCAGAGCUCUUAAUGUU,
SEQ. ID NO. 2307: UGAAGAAUGUCUACAGAUA),

NOS3
(SEQ. ID NO. 2308: UGAAGCACCUGGAGAAUGA,
SEQ. ID NO. 2309: CGGAACAGCACAAGAGUUA,
SEQ. ID NO. 2310: GGAAGAAGACCUUUAAAGA,
SEQ. ID NO. 2309: GCACAAGAGUUAUAAGAUC),

ARH
(SEQ. ID NO. 2310: CGAUACAGCUUGGCACUUU,
SEQ. ID NO. 2311: GAGAAGCGCUGCCCUGUGA,
SEQ. ID NO. 2312: GAAUCAUGCUGUUCUCUUU,
SEQ. ID NO. 2313: GGAGUAACCGGACACCUUA),

CYP7A1

(SEQ. ID NO. 2314: UAAGGUGACUCGAGUGUUU,
SEQ. ID NO. 2315: AAACGACACUUUCAUCAAA,
SEQ. ID NO. 2316: GGACUCAAGUUAAAGUAUU,
SEQ. ID NO. 2317: GUAAUGGACUCAAGUUAAA),

FANCA
(SEQ. ID NO. 2318: GGACAUCACUGCCCACUUC,
SEQ. ID NO. 2319: AGAGGAAGAUGUUCACUUA,
SEQ. ID NO. 2320: GAUCGUGGCUCUUCAGGAA,
SEQ. ID NO. 2321: GGACAGAGGCAGAUAAGAA),

FANCG
(SEQ. ID NO. 2322: GCACUAAGCAGCCUUCAUG,
SEQ. ID NO. 2323: GCAAGCAGGUGCCUACAGA,
SEQ. ID NO. 2324: GGAAUUAGAUGCUCCAUUG,
SEQ. ID NO. 2325: GGACAUCUCUGCCAAAGUC),

ALAS
(SEQ. ID NO. 2326: CAAUAUGCCUGGAAACUAU,
SEQ. ID NO. 2327: GGUUAAGACUCACCAGUUC,
SEQ. ID NO. 2328: CAACAGGACUUUAGGUUCA,
SEQ. ID NO. 2329: GCAUAAGAUUGACAUCAUC),

PIGA
(SEQ. ID NO. 2330: GAAAGAGGGCAUAAGGUUA,
SEQ. ID NO. 2331: GGACUGAUCUUUAAACUAU,
SEQ. ID NO. 2332: UCAAAUGGCUUACUUCAUC,
SEQ. ID NO. 2333: UCUAAGAACUGAUGUCUAA), and

factor VIII
(SEQ. ID NO. 2334: GCAAAUAGAUCUCCAUUAC,
SEQ. ID NO. 2335: CCAGAUAUGUCGUUCUUUA,
SEQ. ID NO. 2336: GAAAGGCUGUGCUCUCAAA,
SEQ. ID NO. 2337: GGAGAAACCUGCAUGAAAG,

SEQ. ID NO. 2338: CUUGAAGCCUCCUGAAUUA,
SEQ. ID NO. 2339: GAGGAAGCAUCCAAAGAUU,
SEQ. ID NO. 2340: GAUAGGAGAUACAAACUUU).

Furthermore, rationally designed siRNA or siRNA pools can be directed against genes involved in disorders of the brain and nervous system. Such genes would include, but are not be limited to:

APBB1

(SEQ. ID NO. 2341: CUACGUAGCUCGUGAUAAG,
SEQ. ID NO. 2342: GCAGAGAUGUCCACACGUU,
SEQ. ID NO. 2343: CAUGAGAUCUGCUCUAAGA,
SEQ. ID NO. 2344: GGGCACCUCUGCUGUAUUG),

BACE1

(SEQ. ID NO. 2345: CCACAGAGCAAGUGAUUUA,
SEQ. ID NO. 2346: GCAGAAAGGAGAUCAUUUA,
SEQ. ID NO. 2347: GUAGCAAGAUCUUUACAUA,
SEQ. ID NO. 2348: UGUCAGAGCUUGAUUAGAA),

PSEN1

(SEQ. ID NO. 2349: GAGCUGACAUUGAAAUAUG,
SEQ. ID NO. 2350: GUACAGCUAUUUCUCAUCA,
SEQ. ID NO. 2351: GAGGUUAGGUGAAGUGGUU,
SEQ. ID NO. 2352: GAAAGGGAGUCACAAGACA,
SEQ. ID NO. 2353: GAACUGGAGUGGAGUAGGA,
SEQ. ID NO. 2354: CAGCAGGCAUAUCUCAUUA,
SEQ. ID NO. 2355: UCAAGUACCUCCCUGAAUG),

PSEN2

(SEQ. ID NO. 2356: GCUGGGAAGUGGCUUAAUA,
SEQ. ID NO. 2357: CAUAUUCCCUGCCCUGAUA,
SEQ. ID NO. 2358: GGGAAGUGCUCAAGACCUA,
SEQ. ID NO. 2359: CAUAGAAAGUGACGUGUUA),

MASS1
(SEQ. ID NO. 2360: GGAAGGAGCUGUUAUGAGA,
SEQ. ID NO. 2361: GAAAGGAGAAGCUAAAUUA,
SEQ. ID NO. 2362: GGAGGAAGGUCAAGAUUUA,
SEQ. ID NO. 2363: GGAAAUAGCUGAGAUAAUG,),

ARX
(SEQ. ID NO. 2364: CCAGACGCCUGAUAUUGAA,
SEQ. ID NO. 2365: CAGCACCACUCAAGACCAA,
SEQ. ID NO. 2366: CGCCUGAUAUUGAAGUAAA,
SEQ. ID NO. 2367: CAACAUCCACUCUCUCUUG) and

NNMT
(SEQ. ID NO. 2368: GGGCAGUGCUCCAGUGGUA,
SEQ. ID NO. 2369: GAAAGAGGCUGGCUACACA,
SEQ. ID NO. 2370: GUACAGAAGUGAGACAUAA,
SEQ. ID NO. 2371: GAGGUGAUCUCGCAAAGUU).

In addition, rationally designed siRNA or siRNA pools can be directed against genes involved in hypertension and related disorders. Such genes would include, but are not be limited to:
angiotensin II type 1 receptor
(SEQ. ID NO. 2372: CAAGAAGCCUGCACCAUGU,
SEQ. ID NO. 2373: GCACUUCACUACCAAAUGA,
SEQ. ID NO. 2374: GCACUGGUCCCAAGUAGUA,
SEQ. ID NO. 2375: CCAAAGGGCAGUAAAGUUU,
SEQ. ID NO. 2376: GCUCAGAGGAGGUGUAUUU,
SEQ. ID NO. 2377: GCACUUCACUACCAAAUGA,
SEQ. ID NO. 2378: AAAGGGCAGUAAAGUUU),

AGTR2
(SEQ. ID NO. 2379: GAACAUCUCUGGCAACAAU,
SEQ. ID NO. 2380: GGUGAUAUAUCUCAAAUUG,
SEQ. ID NO. 2381: GCAAGCAUCUUAUAUAGUU,

SEQ. ID NO. 2382: GAACCAGUCUUUCAACUCA), and other related targets.

Example XIII: Validation of Multigene Knockout using Rab5 and Eps

Two or more genes having similar, overlapping functions often leads to genetic redundancy. Mutations that knockout only one of, *e.g.,* a pair of such genes (also referred to as homologs) results in little or no phenotype due to the fact that the remaining intact gene is capable of fulfilling the role of the disrupted counterpart. To fully understand the function of such genes in cellular physiology, it is often necessary to knockout or knockdown both homologs simultaneously. Unfortunately, concomitant knockdown of two or more genes is frequently difficult to achieve in higher organisms (*e.g.* mice) thus it is necessary to introduce new technologies dissect gene function. One such approach to knocking down multiple genes simultaneously is by using siRNA. For example, **Figure 11** showed that rationally designed siRNA directed against a number of genes involved in the clathrin-mediated endocytosis pathway resulted in significant levels of protein reduction (*e.g.* >80%). To determine the effects of gene knockdown on clathrin-related endocytosis, internalization assays were performed using epidermal growth factor and transferrin. Specifically, mouse receptor-grade EGF (Collaborative Research Inc.) and iron-saturated human transferrin (Sigma) were iodinated as described previously (Jiang, X., Huang, F., Marusyk, A. & Sorkin, A. (2003) *Mol Biol Cell* 14, 858-70). HeLa cells grown in 12-well dishes were incubated with ^{125}I-EGF (1 ng/ml) or ^{125}I-transferrin (1 μg/ml) in binding medium (DMEM, 0.1% bovine serum albumin) at 37°C, and the ratio of internalized and surface radioactivity was determined during 5-min time course to calculate specific internalization rate constant k_e as described previously (Jiang, X *et al.*). The measurements of the uptakes of radiolabeled transferrin and EGF were performed using short time-course assays to avoid influence of the recycling on the uptake kinetics, and using low ligand concentration to avoid saturation of the clathrin-dependent pathway (for EGF Lund, K. A., Opresko, L. K., Strarbuck, C., Walsh, B. J. & Wiley, H. S. (1990) *J. Biol. Chem.* 265, 15713-13723).

The effects of knocking down Rab5a, 5b, 5c, Eps, or Eps 15R (individually) are shown in **Figure 22** and demonstrate that disruption of single genes has little or no effect on EGF or Tfn internalization. In contrast, simultaneous knock down of Rab5a,

5b, and 5c, or Eps and Eps 15R, leads to a distinct phenotype (note: total concentration of siRNA in these experiments remained constant with that in experiments in which a single siRNA was introduced, see **Figure 23**). These experiments demonstrate the effectiveness of using rationally designed siRNA to knockdown multiple genes and validates the utility of these reagents to override genetic redundancy.

Example XIV. Validation of Multigene Targeting Using G6PD, GAPDH, PLK, and UQC.

Further demonstration of the ability to knock down expression of multiple genes using rationally designed siRNA was performed using pools of siRNA directed against four separate genes. To achieve this, siRNA were transfected into cells (total siRNA concentration of 100nM) and assayed twenty-four hours later by B-DNA. Results shown in **Figure 24** show that pools of rationally designed molecules are capable of simultaneously silencing four different genes.

Example XV. Validation of Multigene Knockouts As Demonstrated by Gene Expression Profiling, a Prophetic Example

To further demonstrate the ability to concomitantly knockdown the expression of multiple gene targets, single siRNA or siRNA pools directed against a collection of genes (*e.g.* 4, 8, 16, or 23 different targets) are simultaneously transfected into cells and cultured for twenty-four hours. Subsequently, mRNA is harvested from treated (and untreated) cells and labeled with one of two fluorescent probes dyes (*e.g.* a red fluorescent probe for the treated cells, a green fluorescent probe for the control cells.). Equivalent amounts of labeled RNA from each sample is then mixed together and hybridized to sequences that have been linked to a solid support (*e.g.* a slide, "DNA CHIP"). Following hybridization, the slides are washed and analyzed to assess changes in the levels of target genes induced by siRNA.

Example XVI. Identifying Hyperfunctional siRNA

Identification of Hyperfunctional Bcl-2 siRNA

The ten rationally designed Bcl2 siRNA (identified in **Figure 13, 14**) were tested to identify hyperpotent reagents. To accomplish this, each of the ten Bcl-2

siRNA were individually transfected into cells at a 300pM (0.3nM) concentrations. Twenty-four hours later, transcript levels were assessed by B-DNA assays and compared with relevant controls. As shown in **Figure 25**, while the majority of Bcl-2 siRNA failed to induce functional levels of silencing at this concentration, siRNA 1 and 8 induced >80% silencing, and siRNA 6 exhibited greater than 90% silencing at this subnanomolar concentration.

By way of prophetic examples, similar assays could be performed with any of the groups of rationally designed genes described in Example VII or Example VIII. Thus for instance, rationally designed siRNA sequences directed against

PDGFA

(SEQ. ID NO. 2383: GGUAAGAUAUUGUGCUUUA,
SEQ. ID NO. 2384: CCGCAAAUAUGCAGAAUUA,
SEQ. ID NO. 2385: GGAUGUACAUGGCGUGUUA,
SEQ. ID NO. 2386: GGUGAAGUUUGUAUGUUUA), or

PDGFB

(SEQ. ID NO. 2387: GCUCCGCGCUUUCCGAUUU,
SEQ. ID NO. 2388: GAGCAGGAAUGGUGAGAUG,
SEQ. ID NO. 2389: GAACUUGGGAUAAGAGUGU,
SEQ. ID NO. 2390: CCGAGGAGCUUUAUGAGAU,
SEQ. ID NO. 2391: UUUAUGAGAUGCUGAGUGA)

could be introduced into cells at increasingly limiting concentrations to determine whether any of the duplexes are hyperfunctional. Similarly, rationally designed sequences directed against

HIF1 alpha

(SEQ. ID NO. 2392: GAAGGAACCUGAUGCUUUA,
SEQ. ID NO. 2393: GCAUAUAUCUAGAAGGUAU,
SEQ. ID NO. 2394: GAACAAAUACAUGGGAUUA,
SEQ. ID NO. 2395: GGACACAGAUUUAGACUUG), or

VEGF

(SEQ. ID NO. 2396: GAACGUACUUGCAGAUGUG,
SEQ. ID NO. 2397: GAGAAAGCAUUUGUUUGUA,

SEQ. ID NO. 2398: GGAGAAAGCAUUUGUUUGU,

SEQ. ID NO. 2399: CGAGGCAGCUUGAGUUAAA) could be introduced into cells at increasingly limiting concentrations and screened for hyperfunctional duplexes.

Example XVII: Gene Silencing: Prophetic Example

Below is an example of how one might transfect a cell.

a. Select a cell line. The selection of a cell line is usually determined by the desired application. The most important feature to RNAi is the level of expression of the gene of interest. It is highly recommended to use cell lines for which siRNA transfection conditions have been specified and validated.

b. Plate the cells. Approximately 24 hours prior to transfection, plate the cells at the appropriate density so that they will be approximately 70 – 90% confluent, or approximately 1 x 10^5 cells/ml at the time of transfection. Cell densities that are too low may lead to toxicity due to excess exposure and uptake of transfection reagent-siRNA complexes. Cell densities that are too high may lead to low transfection efficiencies and little or no silencing. Incubate the cells overnight. Standard incubation conditions for mammalian cells are 37°C in 5% CO_2. Other cell types, such as insect cells, require different temperatures and CO_2 concentrations that are readily ascertainable by persons skilled in the art. Use conditions appropriate for the cell type of interest.

c. SiRNA re-suspension: Add 20 µl siRNA universal buffer to each siRNA to generate a final concentration of 50 µM.

d. SiRNA-lipid complex formation. Use RNase-free solutions and tubes. Using the following table, Table XI:

e.

Table XI		
	96-well	24-well
Mixture 1 (TransIT-TKO-Plasmid dilution mixture)		
Opti-MEM	9.3 µl	46.5 µl
TransIT-TKO (1 µg/µl)	0.5 µl	2.5 µl
Mixture 1 Final Volume	**10.0 µl**	**50.0 µl**

Mixture 2 (siRNA dilution mixture)		
Opti-MEM	9.0 µl	45.0 µl
siRNA (1 µM)	1.0 µl	5.0 µl
Mixture 2 Final Volume	**10.0 µl**	**50.0 µl**
Mixture 3 (siRNA-Transfection reagent mixture)		
Mixture 1	10 µl	50 µl
Mixture 2	10 µl	50 µl
Mixture 3 Final Volume	**20 µl**	**100 µl**
Incubate 20 minutes at room temperature.		
Mixture 4 (Media-siRNA/Transfection reagent mixture)		
Mixture 3	20 µl	100 µl
Complete media	80 µl	400 µl
Mixture 4 Final Volume	**100 µl**	**500 µl**
Incubate 48 hours at 37°C.		

Transfection. Create a Mixture 1 by combining the specified amounts of OPTI-MEM serum free media and transfection reagent in a sterile polystyrene tube. Create a Mixture 2 by combining specified amounts of each siRNA with OPTI-MEM media in sterile 1 ml tubes. Create a Mixture 3 by combining specified amounts of Mixture 1 and Mixture 2. Mix gently (do not vortex) and incubate at room temperature for 20 minutes. Create a Mixture 4 by combining specified amounts of Mixture 3 to complete media. Add appropriate volume to each cell culture well. Incubate cells with transfection reagent mixture for 24 – 72 hours at 37°C. This incubation time is flexible. The ratio of silencing will remain consistent at any point in the time period. Assay for gene silencing using an appropriate detection method such as RT-PCR, Western blot analysis, immunohistochemistry, phenotypic analysis, mass

spectrometry, fluorescence, radioactive decay, or any other method that is now known or that comes to be known to persons skilled in the art and that from reading this disclosure would useful with the present invention. The optimal window for observing a knockdown phenotype is related to the mRNA turnover of the gene of interest, although 24 – 72 hours is standard. Final Volume reflects amount needed in each well for the desired cell culture format. When adjusting volumes for a Stock Mix, an additional 10% should be used to accommodate variability in pipetting, *etc.* Duplicate or triplicate assays should be carried out when possible.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departure from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

Claims

1. A method for selecting siRNA comprising selecting an siRNA molecule of 19 – 25 nucleoside bases, said method comprising:
(a) selecting a target gene;
(b) measuring the functionality of sequences of 19 – 25 nucleotides in length that are substantially complementary to a stretch of nucleotides of the target sequence, wherein said functionality is dependent upon non-target specific criteria.

2. The method according to claim 1 wherein said functionality is determined by applying one of the following formulas:

Formula I = $-(GC/3) + (AU_{15\text{-}19}) - (Tm_{20°C})*3 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula II = $-(GC/3) - (AU_{15\text{-}19})*3 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3)$;

Formula III = $-(GC/3) + (AU_{15\text{-}19}) - (Tm_{20°C})*3$;

Formula IV = $-(GC/2) + (AU_{15\text{-}19})/2 - (Tm_{20°C})*2 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula V = $-(G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula VI = $-(G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3)$;

Formula VII = $-(GC/2) + (AU_{15\text{-}19})/2 - (Tm_{20°C})*1 - (G_{13})*3 - (C_{19}) + (A_{19})*3 + (A_3)*3 + (U_{10})/2 + (A_{14})/2 - (U_5)/2 - (A_{11})/2$;

wherein in Formulas I – VII:

$AU_{15\text{-}19}$ = 0 – 5 depending on the number of A or U bases on the sense

strand at positions 15 –19;

G_{13} = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;

C_{19} = 1 if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC= the number of G and C bases in the entire sense strand;

$Tm_{20°C}$ = 1 if the Tm is greater than 20°C;

A_3 = 1 if A is the base at position 3 on the sense strand, otherwise its value is 0;

A_{11} = 1 if A is the base at position 11 on the sense strand, otherwise its value is 0;

A_{14} = 1 if A is the base at position 14 on the sense strand, otherwise its value is 0;

A_{19} = 1 if A is the base at position 19 on the sense strand, otherwise its value is 0;

U_5 = 1 if U is the base at position 5 on the sense strand, otherwise its value is 0;

U_{10} = 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;

or,

Formula VIII: $(-14)*G_{13}-13*A_1-12*U_{5a}-11*U_{5b}-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19}+7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+11*C_9+15*G_1+18*A_3+19*U_{10}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$; and

Formula IX: $(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$

wherein

A_1 = 1 if A is the base at position 1 of the sense strand, otherwise its value is 0;
A_2 = 1 if A is the base at position 2 of the sense strand, otherwise its value is 0;
A_3 = 1 if A is the base at position 3 of the sense strand, otherwise its value is 0;
A_4 = 1 if A is the base at position 4 of the sense strand, otherwise its value is 0;
A_5 = 1 if A is the base at position 5 of the sense strand, otherwise its value is 0;
A_6 = 1 if A is the base at position 6 of the sense strand, otherwise its value is 0;
A_7 = 1 if A is the base at position 7 of the sense strand, otherwise its value is 0;
A_{10} = 1 if A is the base at position 10 of the sense strand, otherwise its value is 0;
A_{11} = 1 if A is the base at position 11 of the sense strand, otherwise its value is 0;
A_{13} = 1 if A is the base at position 13 of the sense strand, otherwise its value is 0;
A_{19} = 1 if A is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

C_3 = 1 if C is the base at position 3 of the sense strand, otherwise its value is 0;
C_4 = 1 if C is the base at position 4 of the sense strand, otherwise its value is 0;
C_5 = 1 if C is the base at position 5 of the sense strand, otherwise its value is 0;
C_6 = 1 if C is the base at position 6 of the sense strand, otherwise its value is 0;
C_7 = 1 if C is the base at position 7 of the sense strand, otherwise its value is 0;
C_9 = 1 if C is the base at position 9 of the sense strand, otherwise its value is 0;
C_{17} = 1 if C is the base at position 17 of the sense strand, otherwise its value is 0;
C_{18} = 1 if C is the base at position 18 of the sense strand, otherwise its value is 0;
C_{19} = 1 if C is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

G_1 = 1 if G is the base at position 1 on the sense strand, otherwise its value is 0;
G_2 = 1 if G is the base at position 2 of the sense strand, otherwise its value is 0;
G_8 = 1 if G is the base at position 8 on the sense strand, otherwise its value is 0;
G_{10} = 1 if G is the base at position 10 on the sense strand, otherwise its value is 0;
G_{13} = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;
G_{19} = 1 if G is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

U_1 = 1 if U is the base at position 1 on the sense strand, otherwise its value is 0;
U_2 = 1 if U is the base at position 2 on the sense strand, otherwise its value is 0;

$U_3 = 1$ if U is the base at position 3 on the sense strand, otherwise its value is 0;
$U_4 = 1$ if U is the base at position 4 on the sense strand, otherwise its value is 0;
$U_7 = 1$ if U is the base at position 7 on the sense strand, otherwise its value is 0;
$U_9 = 1$ if U is the base at position 9 on the sense strand, otherwise its value is 0;
$U_{10} = 1$ if U is the base at position 10 on the sense strand, otherwise its value is 0;
$U_{15} = 1$ if U is the base at position 15 on the sense strand, otherwise its value is 0;
$U_{16} = 1$ if U is the base at position 16 on the sense strand, otherwise its value is 0;
$U_{17} = 1$ if U is the base at position 17 on the sense strand, otherwise its value is 0;
$U_{18} = 1$ if U is the base at position 18 on the sense strand, otherwise its value is 0;

GC_{15-19} = the number of G and C bases within positions 15 – 19 of the sense strand or within positions 15 –18 if the sense strand is only 18 base pairs in length;
GC_{total} = the number of G and C bases in the sense strand;
Tm = 100 if the targeting site contains an inverted repeat longer then 4 base pairs, otherwise its value is 0; and
X = the number of times that the same nucleotide repeats four or more times in a row.

3. A method of gene-silencing comprising selecting an siRNA according to claim 2 and introducing it into a cell.

4. The method according to claim 3 wherein said introducing is by allowing passive uptake of the siRNA.

5. The method according to claim 3, wherein said introducing is through the use of a vector.

6. A method for developing an siRNA algorithm for selecting siRNA, said method comprising:
(a) selecting a set of siRNA;
(b) measuring the gene silencing ability of each siRNA from said set;
(c) determining the relative functionality of each siRNA;
(d) determining the amount of improved functionality by the presence or absence of at least one variable selected from the group consisting of

the total GC content, melting temperature of the siRNA, GC content at positions 15 –19, the presence or absence of a particular nucleotide at a particular position and the number of times that the same nucleotide repeats within a given sequence; and

(e) developing an algorithm using the information of step (d).

7. A method of selecting an siRNA with improved functionality, said method comprising using the algorithm of claim 6.

8. A method of selecting hyperfunctional siRNA, said method comprising using at least one functional siRNA, wherein at least one said functional siRNA has been selected according to the method of claim 7 and measuring the silencing ability of said at least one functional siRNA, wherein silencing ability is measured at a concentration of less than 1 nanomolar siRNA.

9. An siRNA molecule, wherein said siRNA molecule is effective at silencing Bcl-2.

10. The siRNA molecule of claim 9, wherein said siRNA molecule comprises a sequence substantially similar to a sequence selected from the group consisting of GGGAGAUAGUGAUGAAGUA (SEQ. ID NO. 301); GAAGUACAUCCAUUAUAAG (SEQ. ID NO. 302); GUACGACAACCGGGAGAUA (SEQ. ID NO. 303); AGAUAGUGAUGAAGUACAU (SEQ. ID NO. 304); UGAAGACUCUGCUCAGUUU (SEQ. ID NO. 305); CAUGCGGCCUCUGUUUGA (SEQ. ID NO. 306); UGCGGCCUCUGUUUGAUUU (SEQ. ID NO. 307); GAGAUAGUGAUGAAGUACA (SEQ. ID NO. 308); GGAGAUAGUGAUGAAGUAC (SEQ. ID NO. 309); and GAAGACUCUGCUCAGUUUG (SEQ. ID NO. 310).

11. The siRNA molecule of claim 10, wherein said siRNA molecule comprises a sequence selected from the group consisting of

GGGAGAUAGUGAUGAAGUA (SEQ. ID NO. 301);
GAAGUACAUCCAUUAUAAG (SEQ. ID NO. 302);
GUACGACAACCGGGAGAUA (SEQ. ID NO. 303);
AGAUAGUGAUGAAGUACAU (SEQ. ID NO. 304);
UGAAGACUCUGCUCAGUUU (SEQ. ID NO. 305);
GCAUGCGGCCUCUGUUUGA (SEQ. ID NO. 306);
UGCGGCCUCUGUUUGAUUU (SEQ. ID NO. 307);
GAGAUAGUGAUGAAGUACA (SEQ. ID NO. 308);
GGAGAUAGUGAUGAAGUAC (SEQ. ID NO. 309); and
GAAGACUCUGCUCAGUUUG (SEQ. ID NO. 310).

12. The siRNA molecule of claim 11, wherein said siRNA molecule comprises GCAUGCGGCCUCUGUUUGA .

13. The siRNA molecule of claim 9, wherein said siRNA molecule comprises a sense strand and an anti-sense strand.

14. The siRNA molecule of claim 9, wherien said siRNA molecule comprises a hairpin.

15. The siRNA molecule of claim 9, wherein said siRNA molecule comprises between 18 and 30 base pairs.

16. A kit for gene silencing comprising at least one siRNA selected from the group consisting of sequences substantially similar to the group consisting of GGGAGAUAGUGAUGAAGUA (SEQ. ID NO. 301);
GAAGUACAUCCAUUAUAAG (SEQ. ID NO. 302);
GUACGACAACCGGGAGAUA (SEQ. ID NO. 303);
AGAUAGUGAUGAAGUACAU (SEQ. ID NO. 304);
UGAAGACUCUGCUCAGUUU (SEQ. ID NO. 305);
GCAUGCGGCCUCUGUUUGA (SEQ. ID NO. 306);
UGCGGCCUCUGUUUGAUUU (SEQ. ID NO. 307);
GAGAUAGUGAUGAAGUACA (SEQ. ID NO. 308);

GGAGAUAGUGAUGAAGUAC (SEQ. ID NO. 309); and
GAAGACUCUGCUCAGUUUG (SEQ. ID NO. 310).

17. A method of gene silencing comprising using the siRNA molecule of claim 10.

18. A method of gene silencing comprising using the siRNA molecule of claim 11.

19. A kit, wherein said kit is comprised of at least two siRNA, wherein said at least two siRNA comprise a first optimized siRNA and a second optimized siRNA, wherein said first optimized siRNA and said second optimized siRNA are optimized according to one of the following formulas:

Formula I = $-(GC/3) + (AU_{15\text{-}19}) - (Tm_{20°C})*3 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula II = $-(GC/3) - (AU_{15\text{-}19})*3 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3)$;

Formula III = $-(GC/3) + (AU_{15\text{-}19}) - (Tm_{20°C})*3$;

Formula IV = $-(GC/2) + (AU_{15\text{-}19})/2 - (Tm_{20°C})*2 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula V = $-(G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula VI = $-(G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3)$;

Formula VII = $-(GC/2) + (AU_{15\text{-}19})/2 - (Tm_{20°C})*1 - (G_{13})*3 - (C_{19}) + (A_{19})*3 + (A_3)*3 + (U_{10})/2 + (A_{14})/2 - (U_5)/2 - (A_{11})/2$;

wherein in Formulas I – VII:

AU_{15-19} = 0 – 5 depending on the number of A or U bases on the sense strand at positions 15 –19;

G_{13} = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;

C_{19} = 1 if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC = the number of G and C bases in the entire sense strand;

$Tm_{20°C}$ = 1 if the Tm is greater than 20°C;

A_3 = 1 if A is the base at position 3 on the sense strand, otherwise its value is 0;

A_{11} = 1 if A is the base at position 11 on the sense strand, otherwise its value is 0;

A_{14} = 1 if A is the base at position 14 on the sense strand, otherwise its value is 0;

A_{19} = 1 if A is the base at position 19 on the sense strand, otherwise its value is 0;

U_5 = 1 if U is the base at position 5 on the sense strand, otherwise its value is 0;

U_{10} = 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;

or,

Formula VIII: $(-14)*G_{13}-13*A_1-12*U_7-11*U_2-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19}+7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+11*C_9+15*G_1+18*A_3+19*U_{10}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$; and

Formula IX: $(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+$

$(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$

wherein

$A_1 = 1$ if A is the base at position 1 of the sense strand, otherwise its value is 0;
$A_2 = 1$ if A is the base at position 2 of the sense strand, otherwise its value is 0;
$A_3 = 1$ if A is the base at position 3 of the sense strand, otherwise its value is 0;
$A_4 = 1$ if A is the base at position 4 of the sense strand, otherwise its value is 0;
$A_5 = 1$ if A is the base at position 5 of the sense strand, otherwise its value is 0;
$A_6 = 1$ if A is the base at position 6 of the sense strand, otherwise its value is 0;
$A_7 = 1$ if A is the base at position 7 of the sense strand, otherwise its value is 0;
$A_{10} = 1$ if A is the base at position 10 of the sense strand, otherwise its value is 0;
$A_{11} = 1$ if A is the base at position 11 of the sense strand, otherwise its value is 0;
$A_{13} = 1$ if A is the base at position 13 of the sense strand, otherwise its value is 0;
$A_{19} = 1$ if A is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$C_3 = 1$ if C is the base at position 3 of the sense strand, otherwise its value is 0;
$C_4 = 1$ if C is the base at position 4 of the sense strand, otherwise its value is 0;
$C_5 = 1$ if C is the base at position 5 of the sense strand, otherwise its value is 0;
$C_6 = 1$ if C is the base at position 6 of the sense strand, otherwise its value is 0;
$C_7 = 1$ if C is the base at position 7 of the sense strand, otherwise its value is 0;
$C_9 = 1$ if C is the base at position 9 of the sense strand, otherwise its value is 0;
$C_{17} = 1$ if C is the base at position 17 of the sense strand, otherwise its value is 0;
$C_{18} = 1$ if C is the base at position 18 of the sense strand, otherwise its value is 0;
$C_{19} = 1$ if C is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$G_1 = 1$ if G is the base at position 1 on the sense strand, otherwise its value is 0;
$G_2 = 1$ if G is the base at position 2 of the sense strand, otherwise its value is 0;
$G_8 = 1$ if G is the base at position 8 on the sense strand, otherwise its value is 0;
$G_{10} = 1$ if G is the base at position 10 on the sense strand, otherwise its value is 0;
$G_{13} = 1$ if G is the base at position 13 on the sense strand, otherwise its value is 0;

G_{19} = 1 if G is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

U_1 = 1 if U is the base at position 1 on the sense strand, otherwise its value is 0;
U_2 = 1 if U is the base at position 2 on the sense strand, otherwise its value is 0;
U_3 = 1 if U is the base at position 3 on the sense strand, otherwise its value is 0;
U_4 = 1 if U is the base at position 4 on the sense strand, otherwise its value is 0;
U_7 = 1 if U is the base at position 7 on the sense strand, otherwise its value is 0;
U_9 = 1 if U is the base at position 9 on the sense strand, otherwise its value is 0;
U_{10} = 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;
U_{15} = 1 if U is the base at position 15 on the sense strand, otherwise its value is 0;
U_{16} = 1 if U is the base at position 16 on the sense strand, otherwise its value is 0;
U_{17} = 1 if U is the base at position 17 on the sense strand, otherwise its value is 0;
U_{18} = 1 if U is the base at position 18 on the sense strand, otherwise its value is 0;

GC_{15-19} = the number of G and C bases within positions 15 – 19 of the sense strand or within positions 15 –18 if the sense strand is only 18 base pairs in length;
GC_{total} = the number of G and C bases in the sense strand;
Tm = 100 if the targeting site contains an inverted repeat longer then 4 base pairs, otherwise its value is 0; and
X = the number of times that the same nucleotide repeats four or more times in a row.

Figure 1

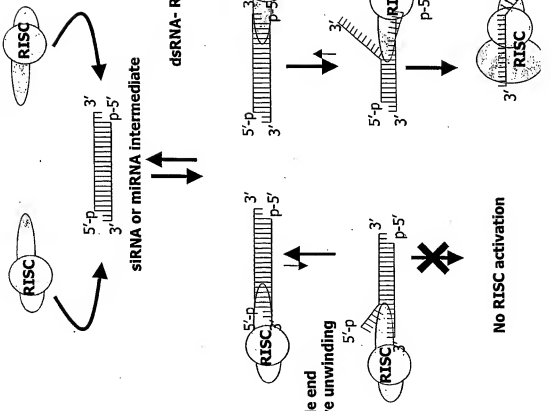

RISC
5'-p
3'
3'
p-5'
siRNA or miRNA intermediate
Reversible
dsRNA- RISC complex formation
5' stable end
Non-processive unwinding
No RISC activation
5' unstable end
Processive unwinding
Irreversible
ssRNA-RISC complex formation

Figure 2

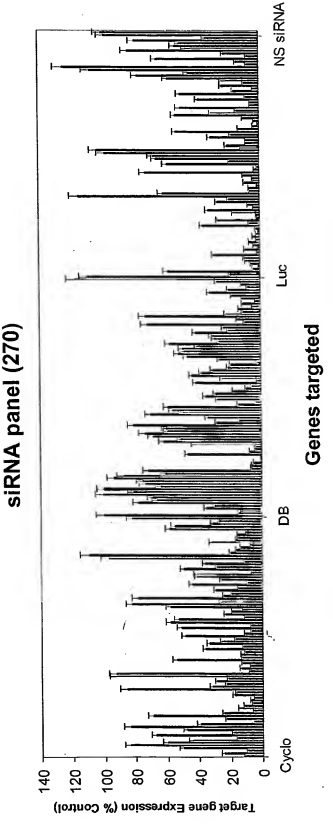
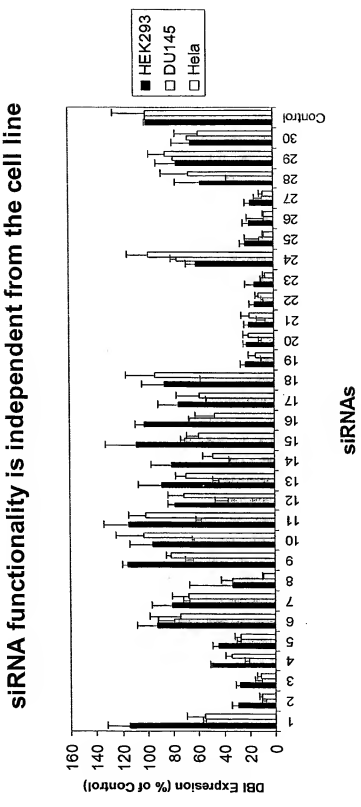

siRNA panel (270)
Target gene Expression (% Control)
140
120
100
80
60
40
20
0
Cyclo
DB
Luc
NS siRNA
Genes targeted

Figure 3a


siRNA functionality is independent from the cell line
HEK293
DU145
Hela
DBI Expression (% of Control)
0
20
40
60
80
100
120
140
160
siRNAs
Control

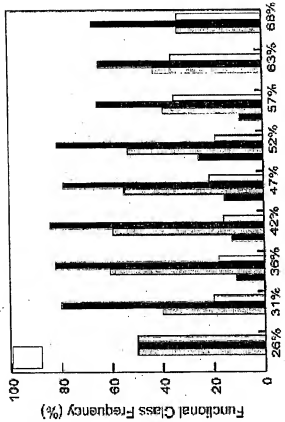


Figure 3b

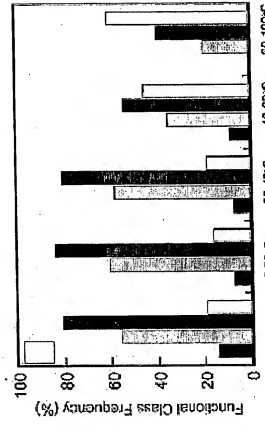


Figure 3c

Figure 4

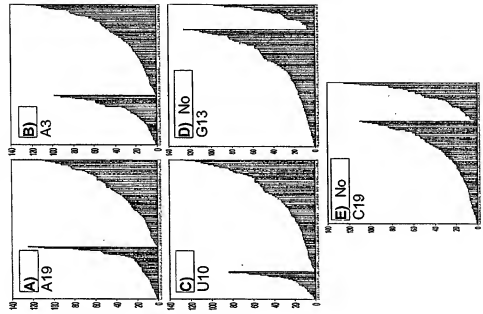


Figure 5A

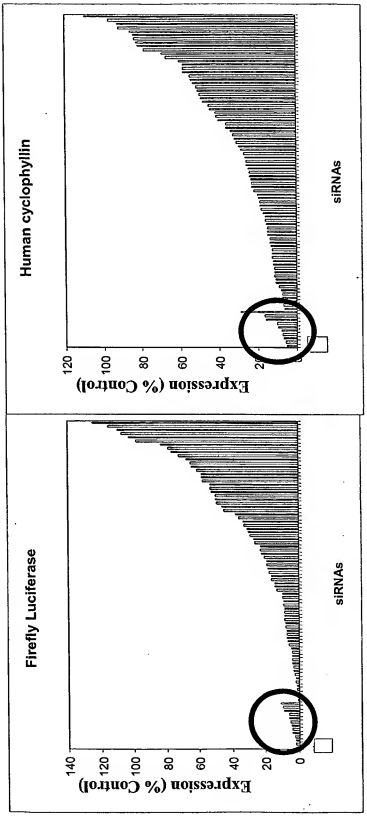


Figure 5B

Figure 6a

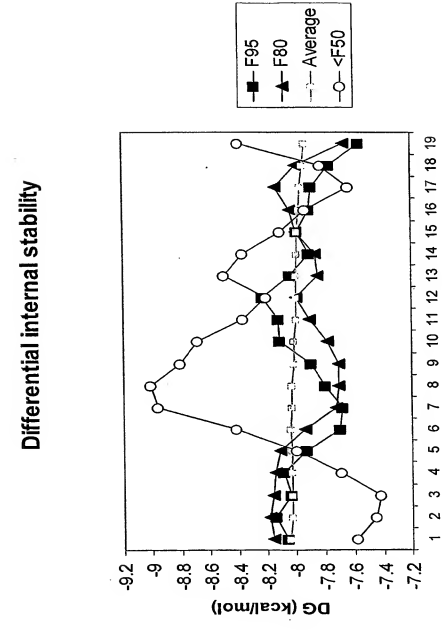

Differential internal stability
DG (kcal/mol)
-9.2
-9
-8.8
-8.6
-8.4
-8.2
-8
-7.8
-7.6
-7.4
-7.2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
5'Sense position
F95
F80
Average
<F50

Figure 6b

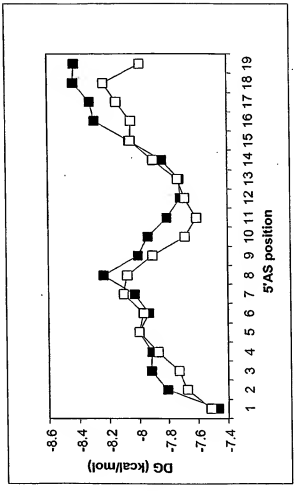
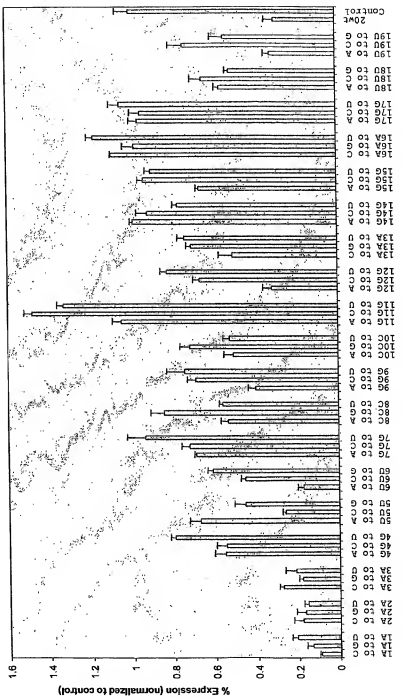

DG (kcal/mol)
-8.6
-8.4
-8.2
-8
-7.8
-7.6
-7.4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
5'AS position

Figure 7


% Expression (normalized to control)
siRNA 100nM
20uM
Control

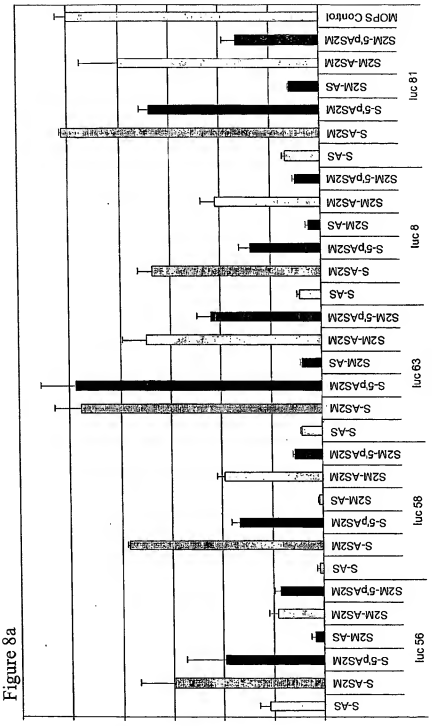

TARGET Screen Normalized LUC ASSAY 293 cells
LUC ASSAY/mock Rate
1.2
1
0.8
0.6
0.4
0.2
0
siRNA 100nM
luc 56
luc 58
luc 63
luc 8
luc 81
S-AS
S-AS2M
S-5'pAS2M
S2M-AS
S2M-AS2M
S2M-5'pAS2M
MOPS Control

Figure 8a

Figure 8b

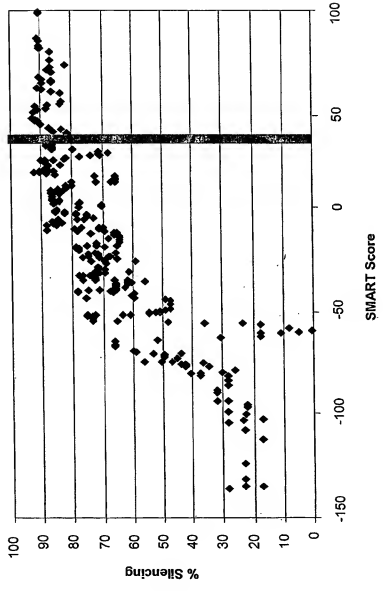

% Silencing
100
90
80
70
60
50
40
30
20
10
0
-150
-100
-50
0
50
100
SMART Score

Figure 9

Figure 10a-f

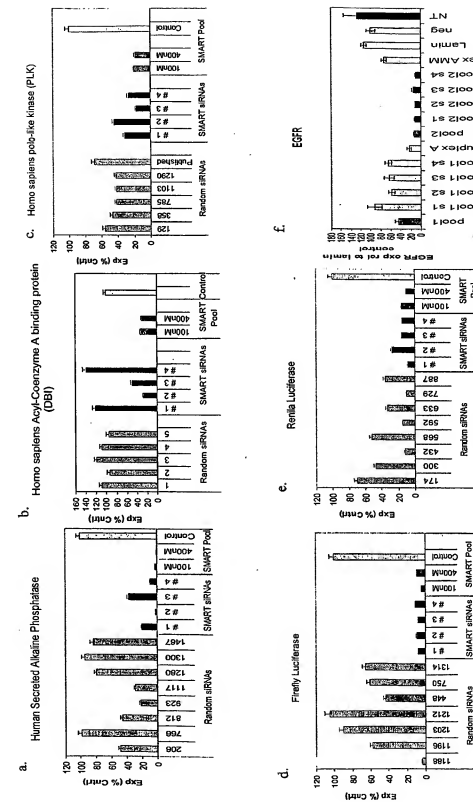

a.
Human Secreted Alkaline Phosphatase
Exp (% Cntrl)
Random siRNAs
SMART siRNAs
SMART Pool
Control
100nM
400nM
b.
Homo sapiens Acyl-Coenzyme A binding protein (DBI)
Exp (% Cntrl)
Random siRNAs
SMART siRNAs
SMART Pool
Control
100nM
400nM
c.
Homo sapiens polo-like kinase (PLK)
Exp (% Cntrl)
Random siRNAs
SMART siRNAs
SMART Pool
Control
100nM
400nM
d.
Firefly Luciferase
Exp (% Cntrl)
Random siRNAs
SMART siRNAs
SMART Pool
Control
100nM
400nM
e.
Renilla Luciferase
Exp (% Cntrl)
Random siRNAs
SMART siRNAs
SMART Pool
Control
100nM
400nM
f
EGFR
EGFR exp rel to lamin control
Duplex applied
pool1
pool1 s1
pool1 s2
pool1 s3
pool1 s4
Duplex A
pool2
pool2 s1
pool2 s2
pool2 s3
pool2 s4
Duplex AMM
Lamin
neg
NT

Figure 11

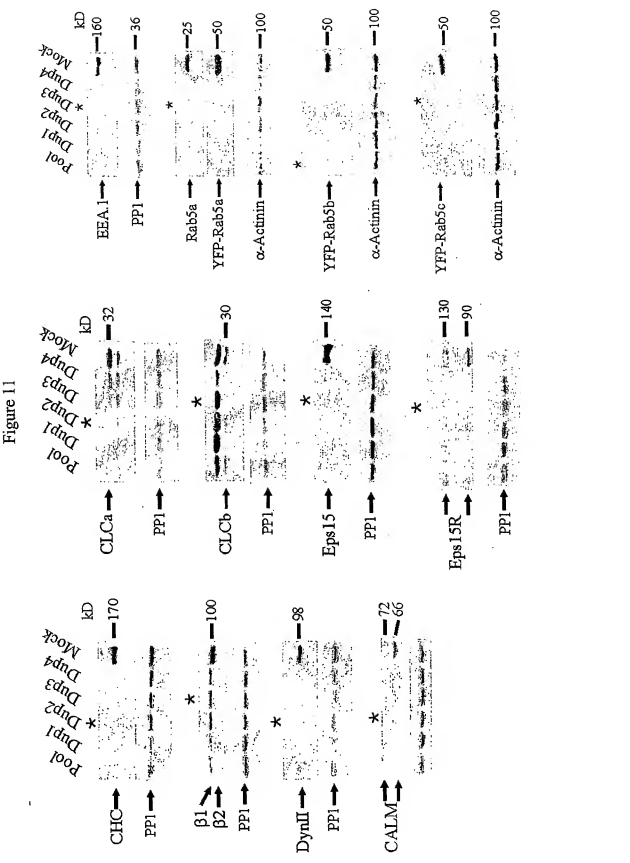

Pool
Dup1
Dup2
Dup3
Dup4
Mock
kD
CHC
170
PP1
β1
β2
100
98
DynII
72
66
CALM
CLCa
32
CLCb
30
Eps15
140
Eps15R
130
90
EEA.1
160
36
Rab5a
25
YFP-Rab5a
50
α-Actinin
YFP-Rab5b
YFP-Rab5c

Figure 12

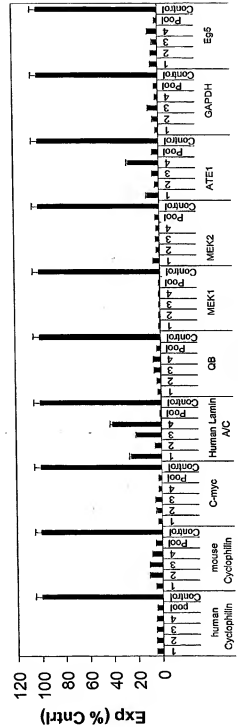
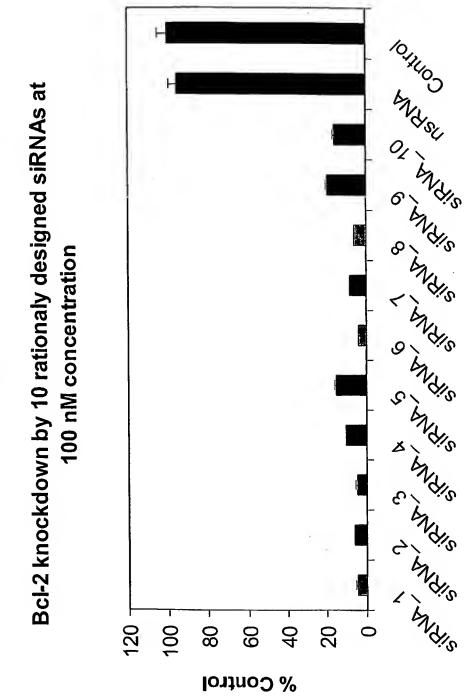

Rational selection validation
Exp (% Cntrl)
120
100
80
60
40
20
0
siRNAs/genes
human Cyclophilin
mouse Cyclophilin
C-myc
Human Lamin A/C
QB
MEK1
MEK2
ATE1
GAPDH
Eg5
Control
Pool
1
2
3
4

Figure 13 Sequences of top Bcl2

siRNA 1	GGGAGAUAGUGAUGAAGUA
siRNA 2	GAAGUACAUCCAUUAUAAG
siRNA 3	GUACGACAACCGGGAGAUA
siRNA 4	AGAUAGUGAUGAAGUACAU
siRNA 5	UGAAGACUCUGCUCAGUUU
siRNA 6	GCAUGCGGCCUCUGUUUGA
siRNA 7	UGCGGCCUCUGUUUGAUUU
siRNA 8	GAGAUAGUGAUGAAGUACA
siRNA 9	GGAGAUAGUGAUGAAGUAC
siRNA 10	GAAGACUCUGCUCAGUUUG

Figure 14


Bcl-2 knockdown by 10 rationaly designed siRNAs at 100 nM concentration
% Control
120
100
80
60
40
20
0
siRNA_1
siRNA_2
siRNA_3
siRNA_4
siRNA_5
siRNA_6
siRNA_7
siRNA_8
siRNA_9
siRNA_10
nsRNA
Control

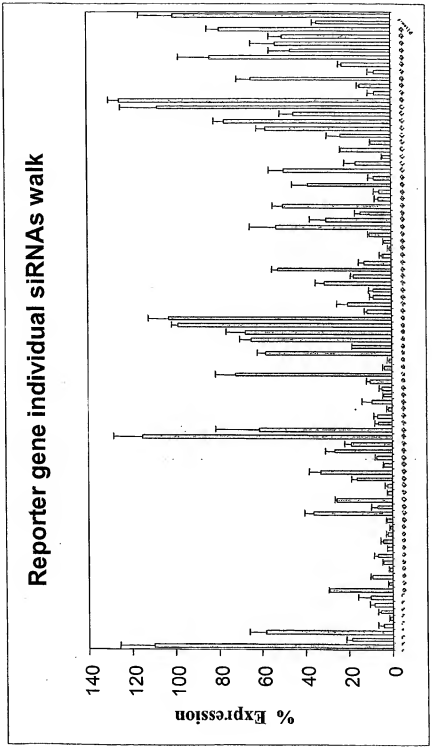

Reporter gene individual siRNAs walk
% Expression
140
120
100
80
60
40
20
0

Figure 15

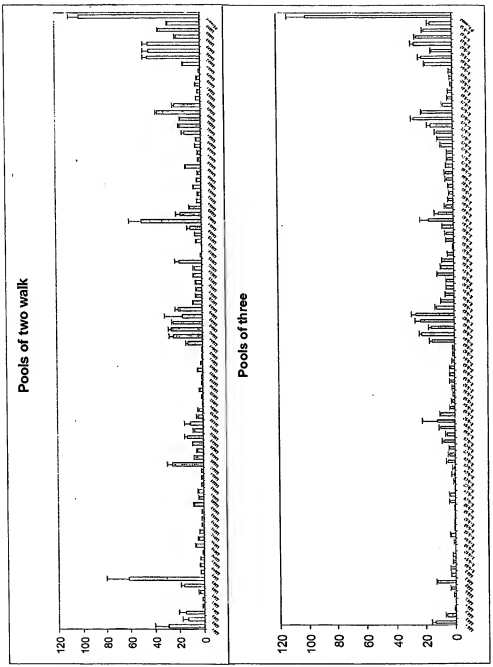

Pools of two walk
Pools of three
% Expression
% Expression
120
100
80
60
40
20
0

Figure 16

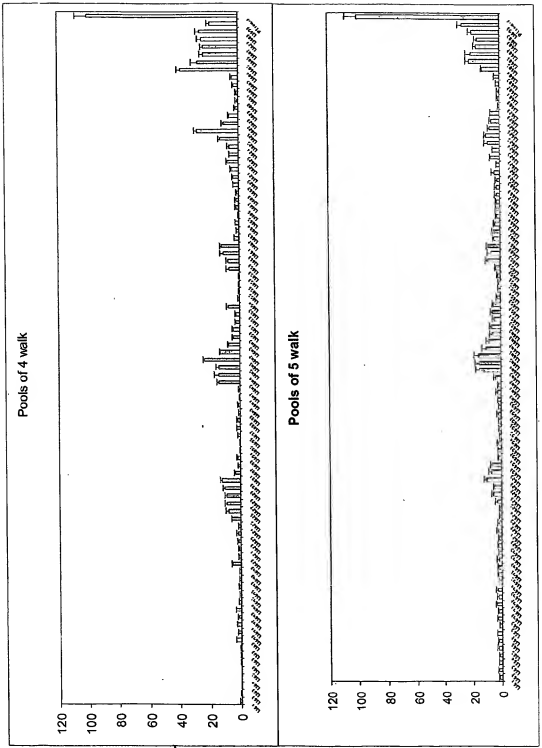

Pools of 4 walk
% Expression
120
100
80
60
40
20
0
Pools of 5 walk
% Expression
120
100
80
60
40
20
0

Figure 17

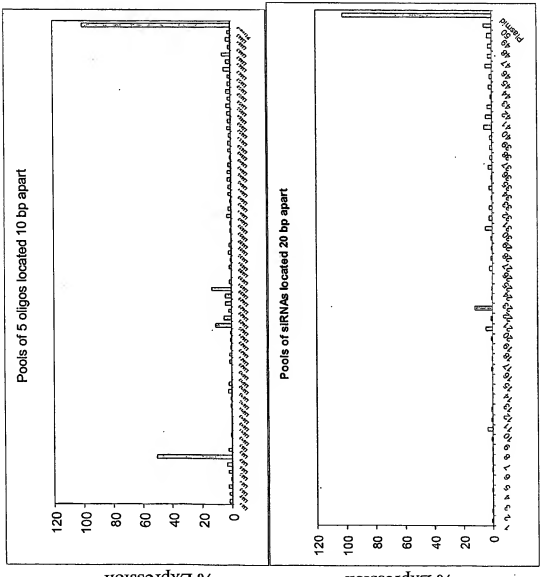

Pools of 5 oligos located 10 bp apart
% Expression
120
100
80
60
40
20
0
Pools of siRNAs located 20 bp apart
% Expression
120
100
80
60
40
20
0
pUBC/Vo

Figure 18

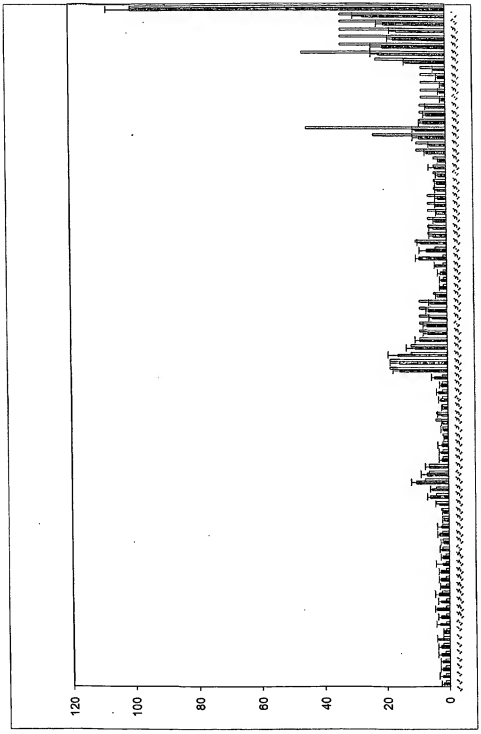


Figure 19

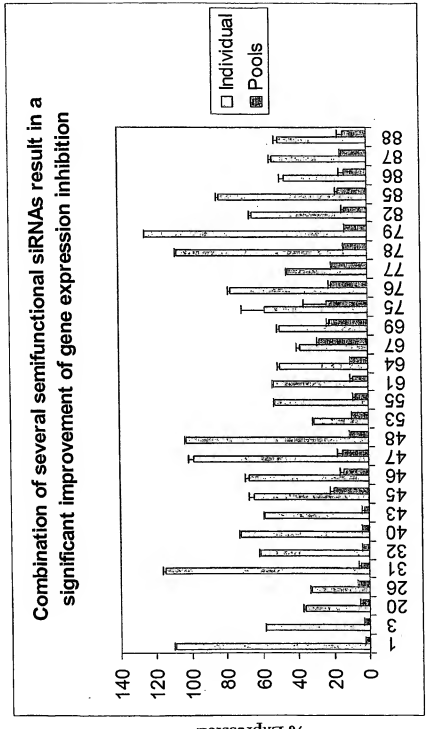

Combination of several semifunctional siRNAs result in a significant improvement of gene expression inhibition
Individual
Pools
% Expression
0
20
40
60
80
100
120
140
1
3
20
26
31
32
40
43
45
46
47
48
53
55
61
64
67
69
75
76
77
78
79
82
85
86
87
88

Figure 20

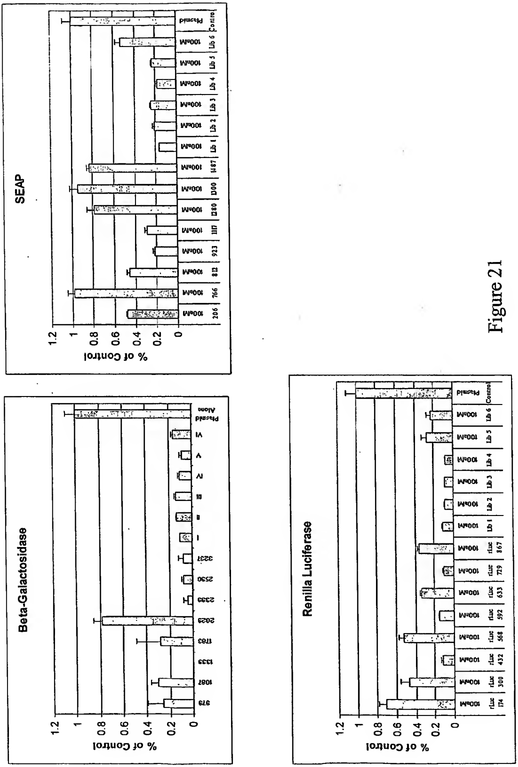

SEAP
% of Control
1.2
1
0.8
0.6
0.4
0.2
0
100nM 206
100nM 766
100nM 812
100nM 923
100nM 1117
100nM 1280
100nM 1300
100nM 1487
100nM Lib 1
100nM Lib 2
100nM Lib 3
100nM Lib 4
100nM Lib 5
100nM Lib 6
Plasmid Control
Beta-Galactosidase
979
1087
1339
1783
2029
2339
2530
3231
I
II
III
IV
V
VI
Plasmid Alone
Renilla Luciferase
100nM rLuc 174
100nM rLuc 300
100nM rLuc 432
100nM rLuc 568
100nM rLuc 592
100nM rLuc 633
100nM rLuc 729
100nM rLuc 867

Figure 21

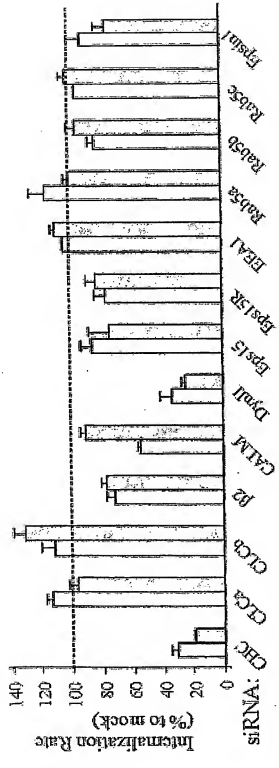

Internalization Rate
(% to mock)
140
120
100
80
60
40
20
0
siRNA:
CHC
CLC a
CLC b
β2
CALM
Dynl
Eps15
Eps15R
EEA1
Rab5a
Rab5b
Rab5c
Epsin1
EGF = □
Tfn = ⊡

Figure 22

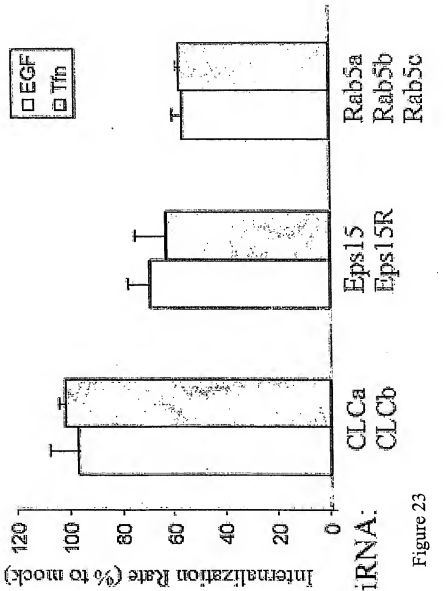

EGF
Tfn
Internalization Rate (% to mock)
120
100
80
60
40
20
0
siRNA:
CLCa
CLCb
Eps15
Eps15R
Rab5a
Rab5b
Rab5c

Figure 23

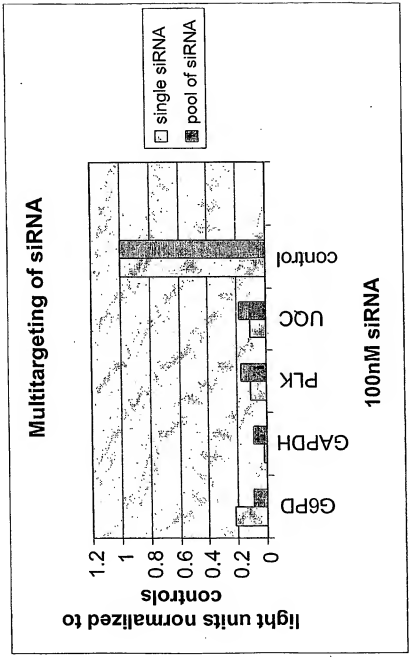
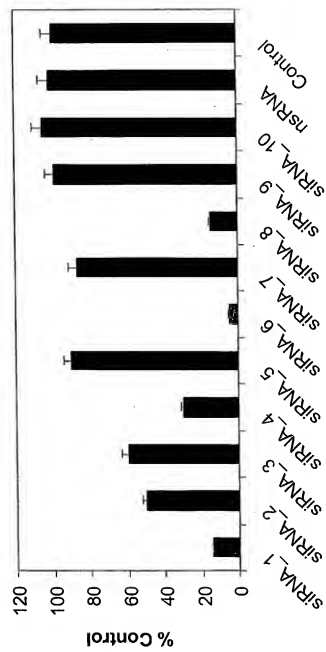

Multitargeting of siRNA
light units normalized to controls
1.2
1
0.8
0.6
0.4
0.2
0
G6PD
GAPDH
PLK
UQC
control
100nM siRNA
single siRNA
pool of siRNA

Figure 24

Figure 25


Bcl-2 knockdown by 10 rationaly designed siRNAs at 300 pM concentration
% Control
120
100
80
60
40
20
0
siRNA_1
siRNA_2
siRNA_3
siRNA_4
siRNA_5
siRNA_6
siRNA_7
siRNA_8
siRNA_9
siRNA_10
nsRNA
Control